1. Release country:

USA

2. Authority overseeing the release:

USDA and EPA

3. Release site:

Seventy-three sites

4. Aim of the release:

Research

5. Duration of post-release monitoring:

One season

6. Aim of post-release monitoring:

Control of potential volunteers

7. Conclusions of post-release monitoring:

The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

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

No adverse effects on human health and the environment observed from the release of 1507 maize

iii. History of previous work relevant to risk assessment prior to commercialization

The product described in this notification is *B.t.* Cry1F maize line 1507, referred to as 1507 maize. It consists of maize product consisting of or derived from seed of 1507 maize genetically modified to express CRY1F protein, conferring resistance to certain lepidopteran insect pests, and PAT protein, conferring tolerance to glufosinate-ammonium herbicide. The maize product also consists of progeny derived from conventional breeding between 1507 maize with any traditionally bred maize.

This notification is for consent to market genetically modified 1507 maize products in accordance with Part C of Directive 2001/18/EC. The scope of this notification is for all uses of 1507 maize including cultivation of 1507 maize seed (inbreds and hybrids) in the EU. The proposed uses of grain and other products of 1507 maize, arising from imports or cultivation, will be the same as for any other maize. The use of 1507 maize for human food is considered in a separate application submitted in accordance with Regulation (EC) No. 258/97.

The risk assessment for 1507 maize has been carried out in a stepwise manner, based on laboratory studies, previous trials in the EU under Part B of Directive 90/220/EEC, similar trials outside the EU, and relevant safety information and studies described in detail throughout this notification and summarized below.

The characteristics of the host organism, maize, have been used as the basis for comparison with 1507 maize. In addition, the origin and function of the genetic material used in the transformation have been evaluated. The molecular characteristics of the genetic modification in 1507 maize have been assessed together with the stability and expression of the introduced traits. Laboratory tests together with toxicity and allergenicity studies have confirmed the specificity and absence of toxicity or allergenicity of CRY1F and PAT proteins as expressed in 1507 maize.

Field trials have confirmed the agronomic performance and expected characteristics of 1507 maize plants. The results show that 1507 maize is comparable to other commercial maize except for the expression of CRY1F and PAT proteins, as intended by the genetic modification.

A detailed environmental risk assessment (e.r.a.) for the placing on the market of 1507 maize for all potential uses including cultivation in the EU has been carried out in accordance to Annex II of Directive 2001/18/EC and relevant guidance notes (Commission Decision 2002/623/EC), and it is attached as **Section 4**. References to previous work relevant to the risk assessment of 1507 maize have been included in the e.r.a. and also throughout the notification, where appropriate. The conclusions from the e.r.a. confirm that there is no risk to human and animal health or the environment arising from the placing on the market of 1507 maize. In addition, there is no significant risk to non-target organisms. However, the e.r.a. does identify a limited potential risk posed by the cultivation of 1507 maize due to the potential development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest

population, and therefore an insect resistance management (IRM) strategy is proposed in the context of product stewardship.

Furthermore, an appropriate monitoring plan in accordance to Annex VII of Directive 2001/18/EC and relevant guidance notes (Commission Decision 2002/811/EC) is attached as **Section 5**. The monitoring plan is considered appropriate as part of the risk management strategy in order to minimize any potential risks from the placing on the market of 1507 maize including cultivation. The monitoring plan has been developed in accordance with the conclusions obtained from the environmental risk assessment contained in this notification and will be applied following approval for the placing on the market of 1507 maize.

The safety evaluation regarding use of 1507 maize for animal feeds in the EU has been described in Annex 1. In addition to the risk assessment contained in this notification for all uses of 1507 maize, a risk assessment is also contained in a separate notification for imports only of 1507 maize as submitted on 23 November 2000 to the Competent Authority of The Netherlands in accordance with Directives 90/220/EEC and 2001/18/EC (Notification No.: C/NL/00/10). An application for approval for food use of 1507 maize has been submitted on 15 February 2001 to the Competent Authority of The Netherlands in accordance with Regulation (EC) No. 258/97.

Furthermore, safety of 1507 maize has been evaluated by the US Environment Protection Agency (EPA), the US Department of Agriculture (USDA) and the US Food and Drug Administration (FDA). The corresponding permits were granted as follows: by US EPA and FDA on 18th May 2001 and by USDA on 14th June 2001.

Additional safety evaluations have been made or are under way by Argentina, Australia/New Zealand, Canada, China, Japan, Korea, Mexico, South Africa, Switzerland and Taiwan. The necessary approvals for import, animal feed use and food safety of 1507 maize in Japan were obtained on 15th June, 28th May and 8th July of 2002, respectively. In Canada permits were granted by Health Canada for novel food use of 1507 maize on 10th October 2002 and by the Canadian Food Inspection Agency for animal feed use and environmental release on 11th October 2002. Approval for import of 1507 maize for animal feed and food use in South Africa was obtained on 12th December 2002.

FIGURES

Figure 1: Restriction map of the 6235 bp insert PHI8999A used in the transformation of 1507 maize

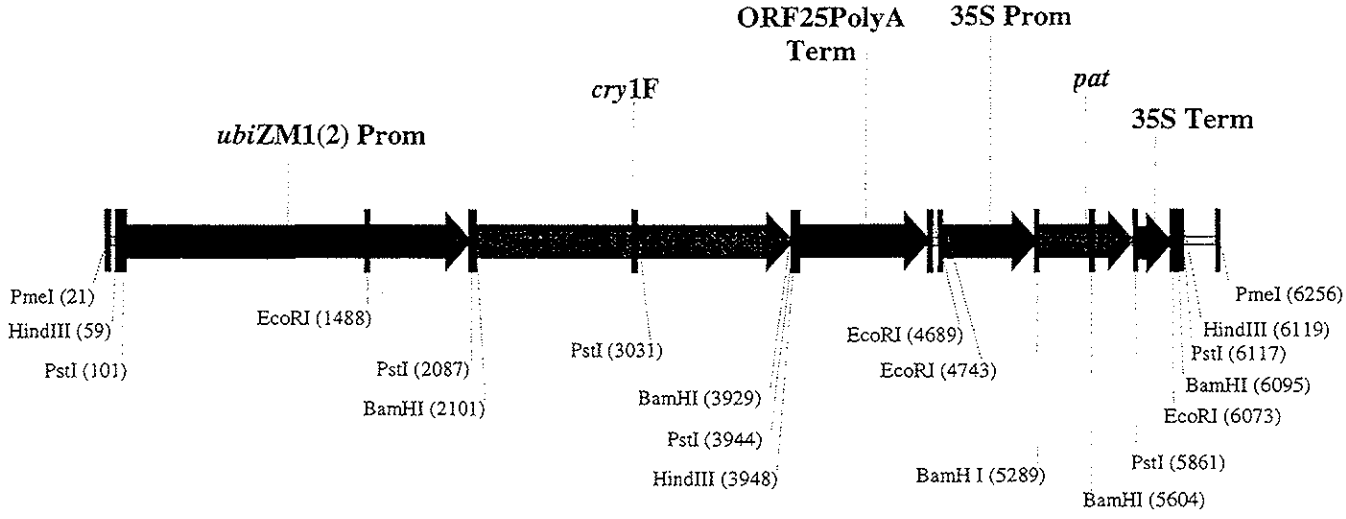

Figure 2: Plasmid map of PHP8999 used in the construction of insert PHI8999A

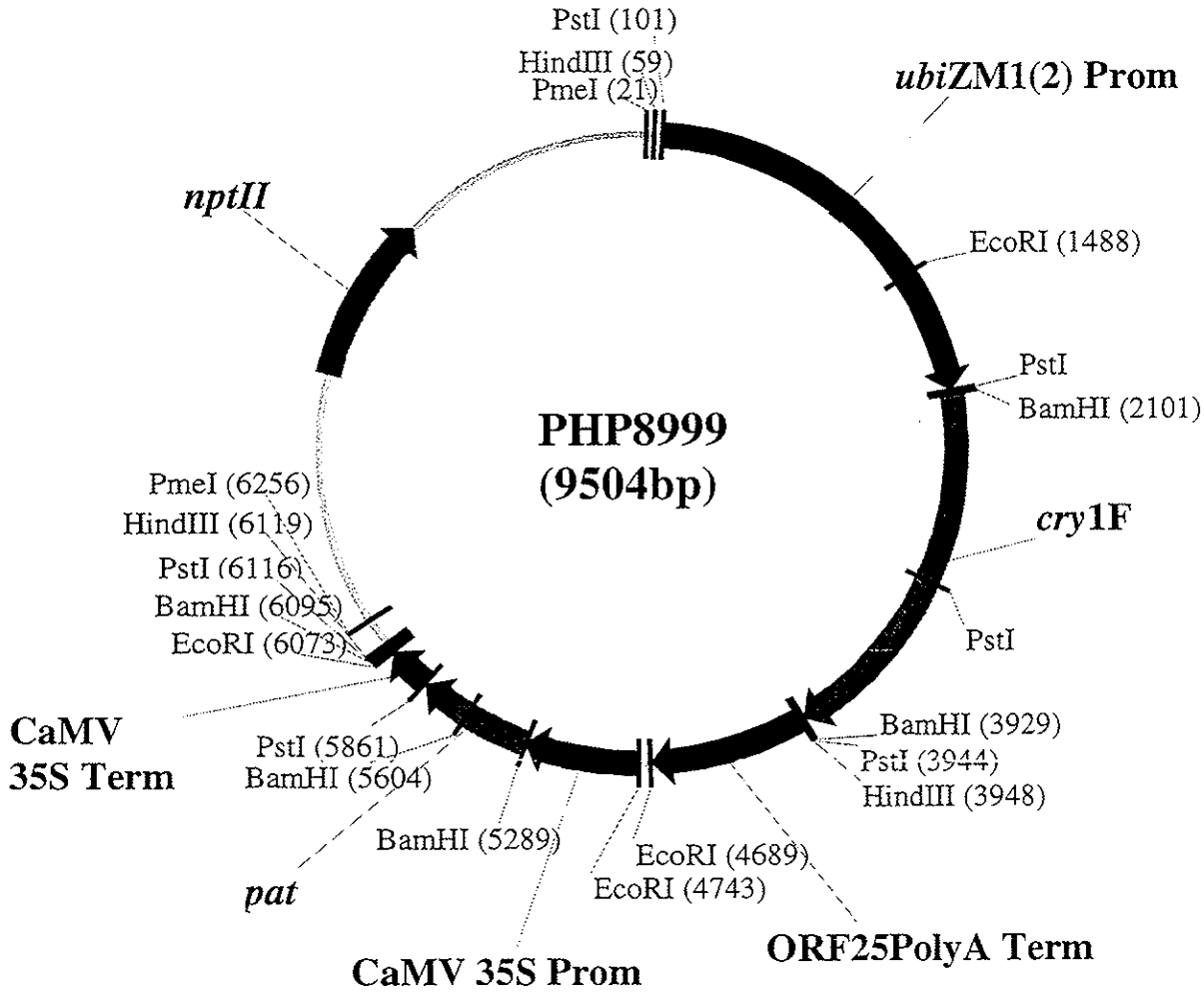

Figure 3: Comparison of the amino acid sequences of microbially-derived CRY1F protein (MR872); CRY1F protein from 1507 maize (CRY1Fsyn); and, CRY1F protein from *Bacillus thuringiensis* sbsp. *aizawai*. The positions of putative protease cleavage sites at the start (about residue 28 or 31) and end (about residue 612 or 615) of the active core toxin are marked with a ↓.

MR872 = Amino acid sequence of microbially-derived CRY1F protein used in toxicological studies
CRY1Fsyn = Amino acid sequence of CRY1F protein from 1507 maize (and from 1360 maize)
CRY1F = Amino acid sequence of CRY1F protein from *B. thuringiensis* sbsp. *aizawai* (Chambers *et al.*, 1991)

```
                                        ↓    ↓
            1                                                    50
    MR872   MENNIQNQCV PYNCLNNPEV EILNEERSTG RLPLDISLSL TRFLLSEFVP
CRY1Fsyn    MENNIQNQCV PYNCLNNPEV EILNEERSTG RLPLDISLSL TRFLLSEFVP
   CRY1F    MENNIQNQCV PYNCLNNPEV EILNEERSTG RLPLDISLSL TRFLLSEFVP
Consensus   MENNIQNQCV PYNCLNNPEV EILNEERSTG RLPLDISLSL TRFLLSEFVP

            51                                                   100
    MR872   GVGVAFGLFD LIWGFITPSD WSLFLLQIEQ LIEQRIETLE RNRAITTLRG
CRY1Fsyn    GVGVAFGLFD LIWGFITPSD WSLFLLQIEQ LIEQRIETLE RNRAITTLRG
   CRY1F    GVGVAFGLFD LIWGFITPSD WSLFLLQIEQ LIEQRIETLE RNRAITTLRG
Consensus   GVGVAFGLFD LIWGFITPSD WSLFLLQIEQ LIEQRIETLE RNRAITTLRG

            101                                                  150
    MR872   LADSYEIYIE ALREWEANPN NAQLREDVRI RFANTDDALI TAINNFTLTS
CRY1Fsyn    LADSYEIYIE ALREWEANPN NAQLREDVRI RFANTDDALI TAINNFTLTS
   CRY1F    LADSYEIYIE ALREWEANPN NAQLREDVRI RFANTDDALI TAINNFTLTS
Consensus   LADSYEIYIE ALREWEANPN NAQLREDVRI RFANTDDALI TAINNFTLTS

            151                                                  200
    MR872   FEIPLLSVYV QAANLHLSLL RDAVSFGQGW GLDIATVNNH YNRLINLIHR
CRY1Fsyn    FEIPLLSVYV QAANLHLSLL RDAVSFGQGW GLDIATVNNH YNRLINLIHR
   CRY1F    FEIPLLSVYV QAANLHLSLL RDAVSFGQGW GLDIATVNNH YNRLINLIHR
Consensus   FEIPLLSVYV QAANLHLSLL RDAVSFGQGW GLDIATVNNH YNRLINLIHR

            201                                                  250
    MR872   YTKHCLDTYN QGLENLRGTN TRQWARFNQF RRDLTLTVLD IVALFPNYDV
CRY1Fsyn    YTKHCLDTYN QGLENLRGTN TRQWARFNQF RRDLTLTVLD IVALFPNYDV
   CRY1F    YTKHCLDTYN QGLENLRGTN TRQWARFNQF RRDLTLTVLD IVALFPNYDV
Consensus   YTKHCLDTYN QGLENLRGTN TRQWARFNQF RRDLTLTVLD IVALFPNYDV

            251                                                  300
    MR872   RTYPIQTSSQ LTREIYTSSV IEDSPVSANI PNGFNRAEFG VRPPHLMDFM
CRY1Fsyn    RTYPIQTSSQ LTREIYTSSV IEDSPVSANI PNGFNRAEFG VRPPHLMDFM
   CRY1F    RTYPIQTSSQ LTREIYTSSV IEDSPVSANI PNGFNRAEFG VRPPHLMDFM
Consensus   RTYPIQTSSQ LTREIYTSSV IEDSPVSANI PNGFNRAEFG VRPPHLMDFM

            301                                                  350
    MR872   NSLFVTAETV RSQTVWGGHL VSSRNTAGNR INFPSYGVFN PGGAIWIADE
CRY1Fsyn    NSLFVTAETV RSQTVWGGHL VSSRNTAGNR INFPSYGVFN PGGAIWIADE
   CRY1F    NSLFVTAETV RSQTVWGGHL VSSRNTAGNR INFPSYGVFN PGGAIWIADE
Consensus   NSLFVTAETV RSQTVWGGHL VSSRNTAGNR INFPSYGVFN PGGAIWIADE

            351                                                  400
    MR872   DPRPFYRTLS DPVFVRGGFG NPHYVLGLRG VAFQQTGTNH TRTFRNSGTI
CRY1Fsyn    DPRPFYRTLS DPVFVRGGFG NPHYVLGLRG VAFQQTGTNH TRTFRNSGTI
   CRY1F    DPRPFYRTLS DPVFVRGGFG NPHYVLGLRG VAFQQTGTNH TRTFRNSGTI
Consensus   DPRPFYRTLS DPVFVRGGFG NPHYVLGLRG VAFQQTGTNH TRTFRNSGTI

            401                                                  450
    MR872   DSLDEIPPQD NSGAPWNDYS HVLNHVTFVR WPGEISGSDS WRAPMFSWTH
CRY1Fsyn    DSLDEIPPQD NSGAPWNDYS HVLNHVTFVR WPGEISGSDS WRAPMFSWTH
   CRY1F    DSLDEIPPQD NSGAPWNDYS HVLNHVTFVR WPGEISGSDS WRAPMFSWTH
Consensus   DSLDEIPPQD NSGAPWNDYS HVLNHVTFVR WPGEISGSDS WRAPMFSWTH

            451                                                  500
    MR872   RSATPTNTID PERITQIPLV KAHTLQSGTT VVRGPGFTGG DILRRTSGGP
CRY1Fsyn    RSATPTNTID PERITQIPLV KAHTLQSGTT VVRGPGFTGG DILRRTSGGP
   CRY1F    RSATPTNTID PERITQIPLV KAHTLQSGTT VVRGPGFTGG DILRRTSGGP
Consensus   RSATPTNTID PERITQIPLV KAHTLQSGTT VVRGPGFTGG DILRRTSGGP
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```
           501                                                        550
    MR872  FAYTIVNING QLPQRYRARI RYASTTNLRI YVTVAGERIF AGQFNKTMDT
 CRY1Fsyn  FAYTIVNING QLPQRYRARI RYASTTNLRI YVTVAGERIF AGQFNKTMDT
    CRY1F  FAYTIVNING QLPQRYRARI RYASTTNLRI YVTVAGERIF AGQFNKTMDT
Consensus  FAYTIVNING QLPQRYRARI RYASTTNLRI YVTVAGERIF AGQFNKTMDT

           551                                                        600
    MR872  GDPLTFQSFS YATINTAFTF PMSQSSFTVG ADTFSSGNEV YIDRFELIPV
 CRY1Fsyn  GDPLTFQSFS YATINTAFTF PMSQSSFTVG ADTFSSGNEV YIDRFELIPV
    CRY1F  GDPLTFQSFS YATINTAFTF PMSQSSFTVG ADTFSSGNEV YIDRFELIPV
Consensus  GDPLTFQSFS YATINTAFTF PMSQSSFTVG ADTFSSGNEV YIDRFELIPV

                       ↓ ↓
           601                                                        650
    MR872  TATFEAEYDL ERAQKAVNAL FTSINQIGIK TDVTDYHIDR VSNLVECLSD
 CRY1Fsyn  TATLE*.... .......... .......... .......... ..........
    CRY1F  TATFEAEYDL ERAQKAVNAL FTSINQIGIK TDVTDYHIDQ VSNLVDCLSD
Consensus  TAT-E----- ---------- ---------- ---------- ----------

           651                                                        700
    MR872  EFCLDEKKEL SEKVKHAKRL SDERNLLQDP NFRGINRQLD RGWRGSTDIT
 CRY1Fsyn  .......... .......... .......... .......... ..........
    CRY1F  EFCLDEKREL SEKVKHAKRL SDERNLLQDP NFKGINRQLD RGWRGSTDIT
Consensus  ---------- ---------- ---------- ---------- ----------


           701                                                        750
    MR872  IQGGDDVFKE NYVTLLGTFD ECYLTYLYQK IDESKLKAYT RYQLRGYIED
 CRY1Fsyn  .......... .......... .......... .......... ..........
    CRY1F  IQRGDDVFKE NYVTLPGTFD ECYPTYLYQK IDESKLKPYT RYQLRGYIED
Consensus  ---------- ---------- ---------- ---------- ----------

           751                                                        800
    MR872  SQDLEIYLIR YNAKHETVNV PGTGSLWRLS APSPI..... ..........
 CRY1Fsyn  .......... .......... .......... .......... ..........
    CRY1F  SQDLEIYLIR YNAKHETVNV LGTGSLWPLS VQSPIRKCGE PNRCAPHLEW
Consensus  ---------- ---------- ---------- ---------- ----------

           801                                                        850
    MR872  .......... .GKCAHHSHH FSLDIDVGCT DLNEDLGVWV IFKIKTQDGH
 CRY1Fsyn  .......... .......... .......... .......... ..........
    CRY1F  NPDLDCSCRD GEKCAHHSHH FSLDIDVGCT DLNEDLDVWV IFKIKTQDGH
Consensus  ---------- ---------- ---------- ---------- ----------

           851                                                        900
    MR872  ARLGNLEFLE EKPLVGEALA RVKRAEKKWR DKREKLEWET NIVYKEAKES
 CRY1Fsyn  .......... .......... .......... .......... ..........
    CRY1F  ARLGNLEFLE EKPLVGEALA RVKRAEKKWR DKREKLELET NIVYKEAKES
Consensus  ---------- ---------- ---------- ---------- ----------

           901                                                        950
    MR872  VDALFVNSQY DRLQADTNIA MIHAADKRVH SIREAYLPEL SVIPGVNAAI
 CRY1Fsyn  .......... .......... .......... .......... ..........
    CRY1F  VDALFVNSQY DQLQADTNIA MIHAADKRVH RIREAYLPEL SVIPGVNVDI
Consensus  ---------- ---------- ---------- ---------- ----------

           951                                                       1000
    MR872  FEELEGRIFT AFSLYDARNV IKNGDFNNGL SCWNVKGHVD VEEQNNHRSV
 CRY1Fsyn  .......... .......... .......... .......... ..........
    CRY1F  FEELKGRIFT AFFLYDARNV IKNGDFNNGL SCWNVKGHVD VEEQNNHRSV
Consensus  ---------- ---------- ---------- ---------- ----------

           1001                                                      1050
    MR872  LVVPEWEAEV SQEVRVCPGR GYILRVTAYK EGYGEGCVTI HEIENNTDEL
 CRY1Fsyn  .......... .......... .......... .......... ..........
    CRY1F  LVVPEWEAEV SQEVRVCPGR GYILRVTAYK EGYGEGCVTI HEIENNTDEL
Consensus  ---------- ---------- ---------- ---------- ----------

           1051                                                      1100
    MR872  KFSNCVEEEV YPNNTVTCND YTATQEEYEG TYTSRNRGYD GAYESNSSVP
 CRY1Fsyn  .......... .......... .......... .......... ..........
    CRY1F  KFSNCVEEEV YPNNTVTCND YTANQEEYGG AYTSRNRGYD ETYGSNSSVP
Consensus  ---------- ---------- ---------- ---------- ----------
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```
           1101                                                      1150
    MR872  ADYASAYEEK AYTDGRRDNP CESNRGYGDY TPLPAGYVTK ELEYFPETDK
 CRY1Fsyn  .......... .......... .......... .......... ..........
    CRY1F  ADYASVYEEK SYTDGRRDNP CESNRGYGDY TPLPAGYVTK ELEYFPETDK
Consensus  ---------- ---------- ---------- ---------- ----------

           1151                  1175
    MR872  VWIEIGETEG TFIVDSVELL LMEE*
 CRY1Fsyn  .......... .......... .....
    CRY1F  VWIEIGETEG TFIVDSVELL LMEE*
Consensus  ---------- ---------- -----
```

Figure 4: Sequence of the *ubi*ZM1(2) promoter from *Zea mays* as present in insert PHI8999A, used in the transformation of 1507 maize (Christensen *et al.*, 1992)

⌈*ubi*ZM1(2) (bp 81 – 2066)

```
                                                    GTG CAGCGTGACC
                                                    CAC GTCGCACTGG

  94   CGGTCGTGCC CCTCTCTAGA GATAATGAGC ATTGCATGTC TAAGTTATAA
       GCCAGCACGG GGAGAGATCT CTATTACTCG TAACGTACAG ATTCAATATT

 144   AAAATTACCA CATATTTTTT TTGTCACACT TGTTTGAAGT GCAGTTTATC
       TTTTAATGGT GTATAAAAAA AACAGTGTGA ACAAACTTCA CGTCAAATAG

 194   TATCTTTATA CATATATTTA AACTTTACTC TACGAATAAT ATAATCTATA
       ATAGAAATAT GTATATAAAT TTGAAATGAG ATGCTTATTA TATTAGATAT

 244   GTACTACAAT AATATCAGTG TTTTAGAGAA TCATATAAAT GAACAGTTAG
       CATGATGTTA TTATAGTCAC AAAATCTCTT AGTATATTTA CTTGTCAATC

 294   ACATGGTCTA AAGGACAATT GAGTATTTTG ACAACAGGAC TCTACAGTTT
       TGTACCAGAT TTCCTGTTAA CTCATAAAAC TGTTGTCCTG AGATGTCAAA

 344   TATCTTTTTA GTGTGCATGT GTTCTCCTTT TTTTTTGCAA ATAGCTTCAC
       ATAGAAAAAT CACACGTACA CAAGAGGAAA AAAAAACGTT TATCGAAGTG

 394   CTATATAATA CTTCATCCAT TTTATTAGTA CATCCATTTA GGGTTTAGGG
       GATATATTAT GAAGTAGGTA AAATAATCAT GTAGGTAAAT CCCAAATCCC

 444   TTAATGGTTT TTATAGACTA ATTTTTTTAG TACATCTATT TTATTCTATT
       AATTACCAAA AATATCTGAT TAAAAAAATC ATGTAGATAA AATAAGATAA

 494   TTAGCCTCTA AATTAAGAAA ACTAAAACTC TATTTTAGTT TTTTTATTTA
       AATCGGAGAT TTAATTCTTT TGATTTTGAG ATAAAATCAA AAAAATAAAT

 544   ATAATTTAGA TATAAAATAG AATAAAATAA AGTGACTAAA AATTAAACAA
       TATTAAATCT ATATTTTATC TTATTTTATT TCACTGATTT TTAATTTGTT

 594   ATACCCTTTA AGAAATTAAA AAAACTAAGG AAACATTTTT CTTGTTTCGA
       TATGGGAAAT TCTTTAATTT TTTTGATTCC TTTGTAAAAA GAACAAAGCT

 644   GTAGATAATG CCAGCCTGTT AAACGCCGTC GACGAGTCTA ACGGACACCA
       CATCTATTAC GGTCGGACAA TTTGCGGCAG CTGCTCAGAT TGCCTGTGGT

 694   ACCAGCGAAC CAGCAGCGTC GCGTCGGGCC AAGCGAAGCA GACGGCACGG
       TGGTCGCTTG GTCGTCGCAG CGCAGCCCGG TTCGCTTCGT CTGCCGTGCC

 744   CATCTCTGTC GCTGCCTCTG GACCCCTCTC GAGAGTTCCG CTCCACCGTT
       GTAGAGACAG CGACGGAGAC CTGGGGAGAG CTCTCAAGGC GAGGTGGCAA

 794   GGACTTGCTC CGCTGTCGGC ATCCAGAAAT TGCGTGGCGG AGCGGCAGAC
       CCTGAACGAG GCGACAGCCG TAGGTCTTTA ACGCACCGCC TCGCCGTCTG

 844   GTGAGCCGGC ACGGCAGGCG GCCTCCTCCT CCTCTCACGG CACGGCAGCT
       CACTCGGCCG TGCCGTCCGC CGGAGGAGGA GGAGAGTGCC GTGCCGTCGA

 894   ACGGGGGATT CCTTTCCCAC CGCTCCTTCG CTTTCCCTTC CTCGCCCGCC
       TGCCCCCTAA GGAAAGGGTG GCGAGGAAGC GAAAGGGAAG GAGCGGGCGG

 944   GTAATAAATA GACACCCCCT CCACACCCTC TTTCCCCAAC CTCGTGTTGT
       CATTATTTAT CTGTGGGGGA GGTGTGGGAG AAAGGGGTTG GAGCACAACA
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 994   TCGGAGCGCA CACACACACA ACCAGATCTC CCCCAAATCC ACCCGTCGGC
       AGCCTCGCGT GTGTGTGTGT TGGTCTAGAG GGGGTTTAGG TGGGCAGCCG

1044   ACCTCCGCTT CAAGGTACGC CGCTCGTCCT CCCCCCCCCC CCCTCTCTAC
       TGGAGGCGAA GTTCCATGCG GCGAGCAGGA GGGGGGGGGG GGGAGAGATG

1094   CTTCTCTAGA TCGGCGTTCC GGTCCATGGT TAGGGCCCGG TAGTTCTACT
       GAAGAGATCT AGCCGCAAGG CCAGGTACCA ATCCCGGGCC ATCAAGATGA

1144   TCTGTTCATG TTTGTGTTAG ATCCGTGTTT GTGTTAGATC CGTGCTGCTA
       AGACAAGTAC AAACACAATC TAGGCACAAA CACAATCTAG GCACGACGAT

1194   GCGTTCGTAC ACGGATGCGA CCTGTACGTC AGACACGTTC TGATTGCTAA
       CGCAAGCATG TGCCTACGCT GGACATGCAG TCTGTGCAAG ACTAACGATT

1244   CTTGCCAGTG TTTCTCTTTG GGGAATCCTG GGATGGCTCT AGCCGTTCCG
       GAACGGTCAC AAAGAGAAAC CCCTTAGGAC CCTACCGAGA TCGGCAAGGC

1294   CAGACGGGAT CGATTTCATG ATTTTTTTTG TTTCGTTGCA TAGGGTTTGG
       GTCTGCCCTA GCTAAAGTAC TAAAAAAAAC AAAGCAACGT ATCCCAAACC

1344   TTTGCCCTTT TCCTTTATTT CAATATATGC CGTGCACTTG TTTGTCGGGT
       AAACGGGAAA AGGAAATAAA GTTATATACG GCACGTGAAC AAACAGCCCA

1394   CATCTTTTCA TGCTTTTTTT TGTCTTGGTT GTGATGATGT GGTCTGGTTG
       GTAGAAAAGT ACGAAAAAAA ACAGAACCAA CACTACTACA CCAGACCAAC

1444   GGCGGTCGTT CTAGATCGGA GTAGAATTCT GTTTCAAACT ACCTGGTGGA
       CCGCCAGCAA GATCTAGCCT CATCTTAAGA CAAAGTTTGA TGGACCACCT

1494   TTTATTAATT TTGGATCTGT ATGTGTGTGC CATACATATT CATAGTTACG
       AAATAATTAA AACCTAGACA TACACACACG GTATGTATAA GTATCAATGC

1544   AATTGAAGAT GATGGATGGA AATATCGATC TAGGATAGGT ATACATGTTG
       TTAACTTCTA CTACCTACCT TTATAGCTAG ATCCTATCCA TATGTACAAC

1594   ATGCGGGTTT TACTGATGCA TATACAGAGA TGCTTTTTGT TCGCTTGGTT
       TACGCCCAAA ATGACTACGT ATATGTCTCT ACGAAAAACA AGCGAACCAA

1644   GTGATGATGT GGTGTGGTTG GGCGGTCGTT CATTCGTTCT AGATCGGAGT
       CACTACTACA CCACACCAAC CCGCCAGCAA GTAAGCAAGA TCTAGCCTCA

1694   AGAATACTGT TTCAAACTAC CTGGTGTATT TATTAATTTT GGAACTGTAT
       TCTTATGACA AAGTTTGATG GACCACATAA ATAATTAAAA CCTTGACATA

1744   GTGTGTGTCA TACATCTTCA TAGTTACGAG TTTAAGATGG ATGGAAATAT
       CACACACAGT ATGTAGAAGT ATCAATGCTC AAATTCTACC TACCTTTATA

1794   CGATCTAGGA TAGGTATACA TGTTGATGTG GGTTTTACTG ATGCATATAC
       GCTAGATCCT ATCCATATGT ACAACTACAC CCAAAATGAC TACGTATATG

1844   ATGATGGCAT ATGCAGCATC TATTCATATG CTCTAACCTT GAGTACCTAT
       TACTACCGTA TACGTCGTAG ATAAGTATAC GAGATTGGAA CTCATGGATA

1894   CTATTATAAT AAACAAGTAT GTTTTATAAT TATTTTGATC TTGATATACT
       GATAATATTA TTTGTTCATA CAAAATATTA ATAAAACTAG AACTATATGA

1944   TGGATGATGG CATATGCAGC AGCTATATGT GGATTTTTTT AGCCCTGCCT
       ACCTACTACC GTATACGTCG TCGATATACA CCTAAAAAAA TCGGGACGGA
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1954    TCATACGCTA TTTATTTGCT TGGTACTGTT TCTTTTGTCG ATGCTCACCC
        AGTATGCGAT AAATAAACGA ACCATGACAA AGAAAACAGC TACGAGTGGG
          ubiZM1(2) (bp 81 – 2066)      ]
2044    TGTTGTTTGG TGTTACTTCT GCA
        ACAACAAACC ACAATGAAGA CGT
```

Figure 5: Sequence of the ORF25PolyA terminator from *Agrobacterium tumefaciens* as present in insert PHI8999A, used in the transformation of 1507 maize (Barker *et al.*, 1983)

ORF25 polyA terminator (bp 3954 – 4667)

```
                    GACATATGCC CCGGTTTCGT TGCGACTAAC ATGAGTTCTT
                    CTGTATACGG GGCCAAAGCA ACGCTGATTG TACTCAAGAA

3994    GGACAAATTT GATTGGACCT GATGAGATGA TCCAACCCGA GGATATAGCA
        CCTGTTTAAA CTAACCTGGA CTACTCTACT AGGTTGGGCT CCTATATCGT

4044    AAGCTCGTTC GTGCAGCAAT GGAACGGCCA AACCGTGCTT TTGTCCCCAA
        TTCGAGCAAG CACGTCGTTA CCTTGCCGGT TTGGCACGAA AACAGGGGTT

4094    GAATGAGGTG CTATGCATGA AGGAATCTAC CCGTTGATGT CCAACAGTCT
        CTTACTCCAC GATACGTACT TCCTTAGATG GGCAACTACA GGTTGTCAGA

4144    CAGGGTTAAT GTCTATGTAT CTTAAATAAT GTTGTCGGTA TTTTGTAATC
        GTCCCAATTA CAGATACATA GAATTTATTA CAACAGCCAT AAAACATTAG

4194    TCATATAGAT TTTCACTGTG CGACGCAAAA ATATTAAATA AATATTATTA
        AGTATATCTA AAAGTGACAC GCTGCGTTTT TATAATTTAT TTATAATAAT

4244    TTATCTACGT TTTGATTGAG ATATCATCAA TATTATAATA AAAATATCCA
        AATAGATGCA AAACTAACTC TATAGTAGTT ATAATATTAT TTTTATAGGT

4294    TTAAACACGA TTTGATACAA ATGACAGTCA ATAATCTGAT TTGAATATTT
        AATTTGTGCT AAACTATGTT TACTGTCAGT TATTAGACTA AACTTATAAA

4344    ATTAATTGTA ACGAATTACA TAAAGATCGA ATAGAAAATA CTGCACTGCA
        TAATTAACAT TGCTTAATGT ATTTCTAGCT TATCTTTTAT GACGTGACGT

4394    AATGAAAATT AACACATACT AATAAATGCG TCAAATATCT TTGCCAAGAT
        TTACTTTTAA TTGTGTATGA TTATTTACGC AGTTTATAGA AACGGTTCTA

4444    CAAGCGGAGT GAGGGCCTCA TATCCGGTCT CAGTTACAAG CACGGTATCC
        GTTCGCCTCA CTCCCGGAGT ATAGGCCAGA GTCAATGTTC GTGCCATAGG

4494    CCGAAGCGCG CTCCACCAAT GCCCTCGACA TAGATGCCGG GCTCGACGCT
        GGCTTCGCGC GAGGTGGTTA CGGGAGCTGT ATCTACGGCC CGAGCTGCGA

4544    GAGGACATTG CCTACCTTGA GCATGGTCTC AGCGCCGGCT TTAAGCTCAA
        CTCCTGTAAC GGATGGAACT CGTACCAGAG TCGCGGCCGA AATTCGAGTT

4594    TCCCATCCCA ATCTGAATAT CCTATCCCGC GCCCAGTCCG GTGTAAGAAC
        AGGGTAGGGT TAGACTTATA GGATAGGGCG CGGGTCAGGC CACATTCTTG
```

ORF25 polyA terminator (bp 3954 – 4667)•

```
4644    GGGTCTGTCC ATCCACCTCT GTTG
        CCCAGACAGG TAGGTGGAGA CAAC
```

Figure 6: Analysis of PHP8999 plasmid for the presence of possible open reading frames (ORFs), both sense and anti-sense, larger than 300 bp in length. ORFs larger than 100 residues and starting with ATG are indicated by arrows

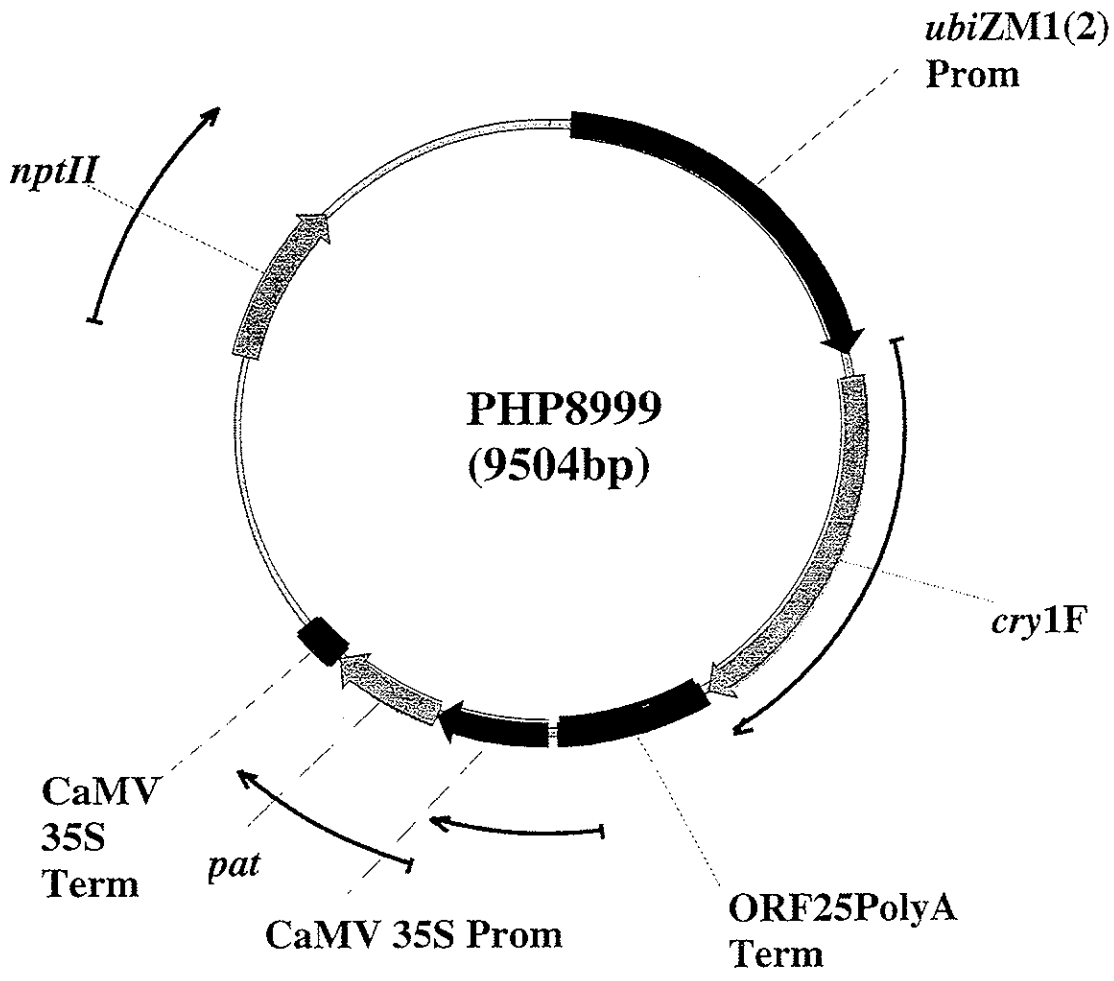


Figure 7: Plasmid map showing the exact locations on PHP8999A of the probes used for the molecular characterization of the genetic material inserted in 1507 maize

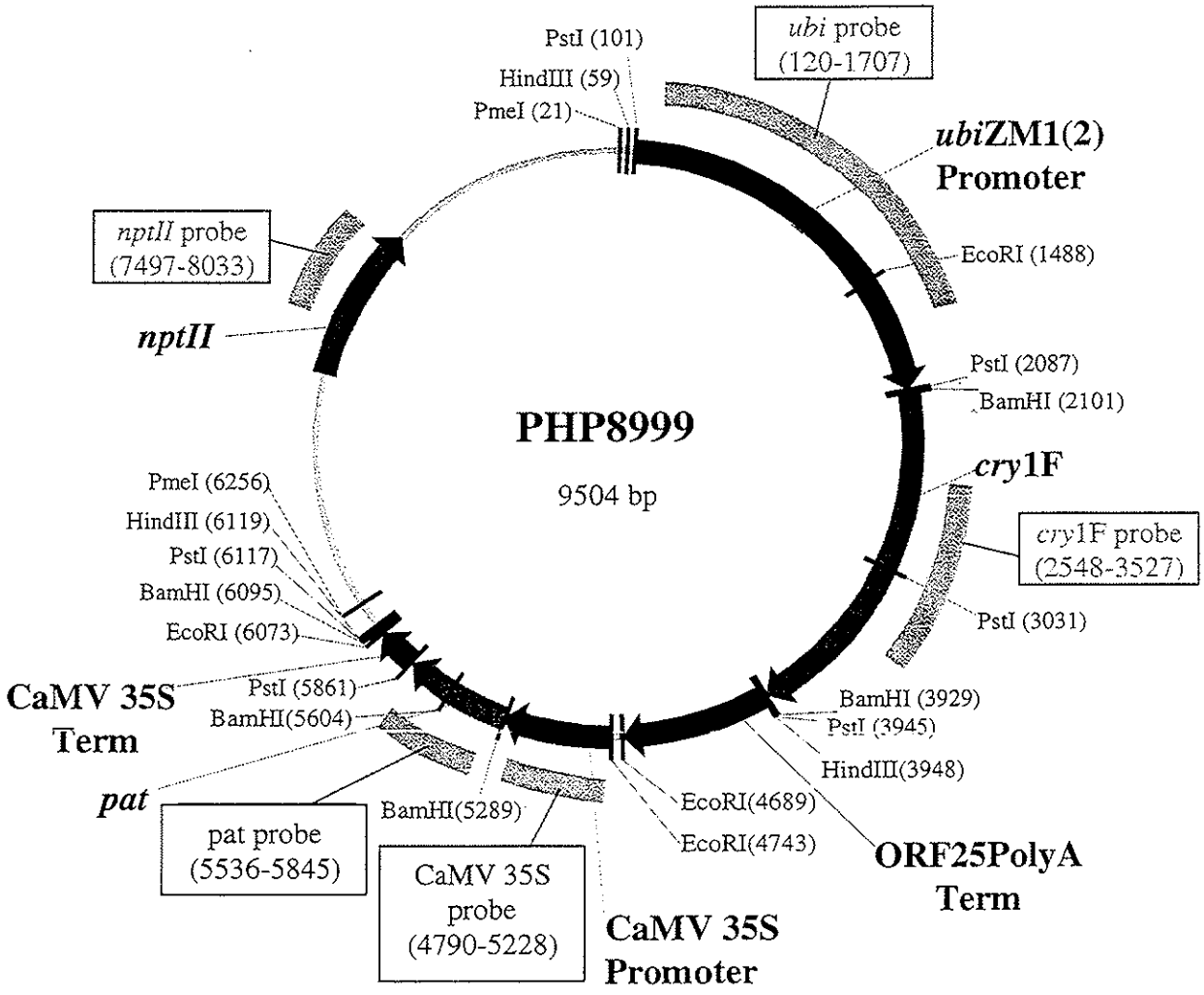
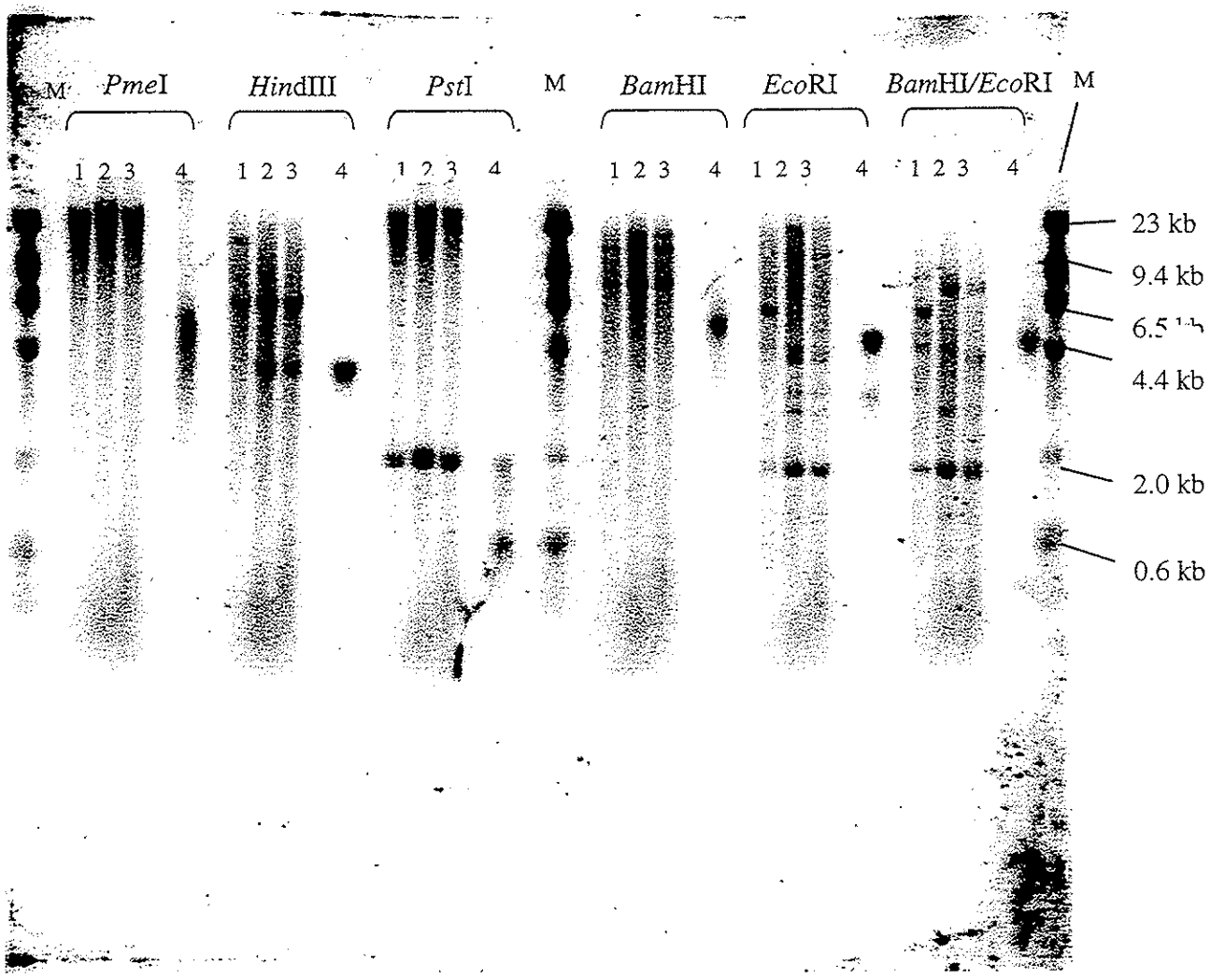
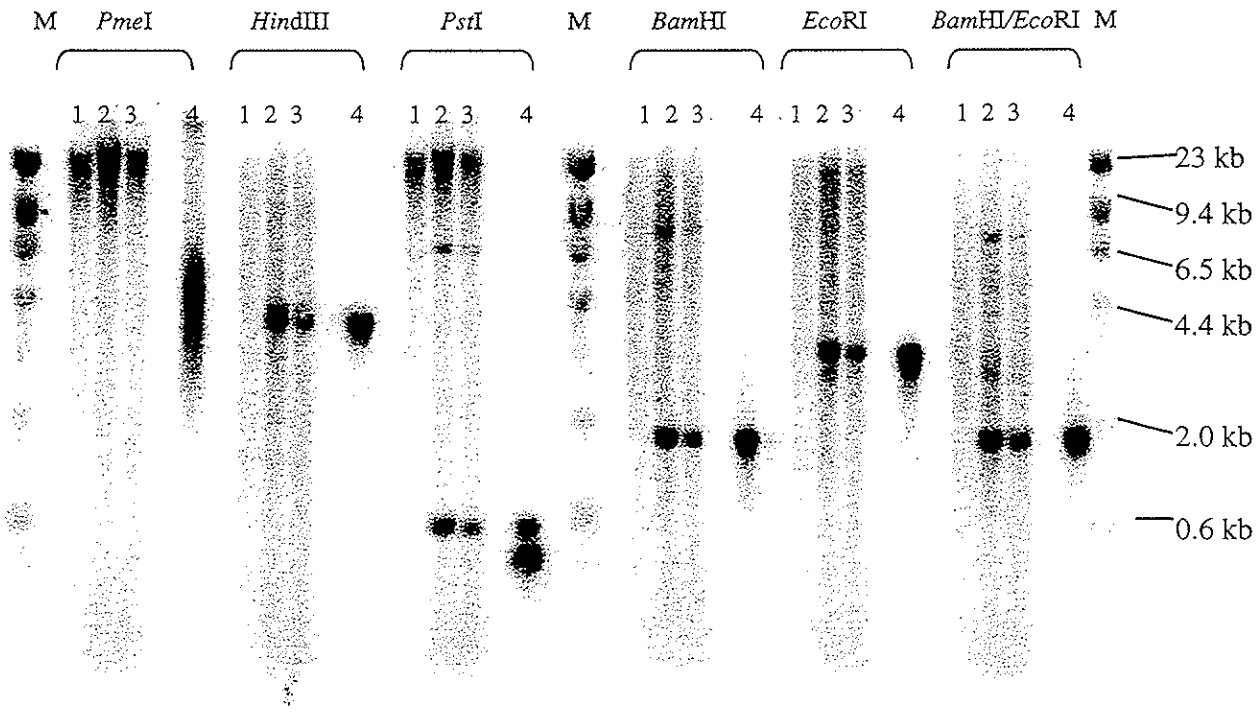


Figure 8: Southern blot analysis of the *ubi*ZM1(2) promoter for the *cry*1F gene in the DNA insert of 1507 maize



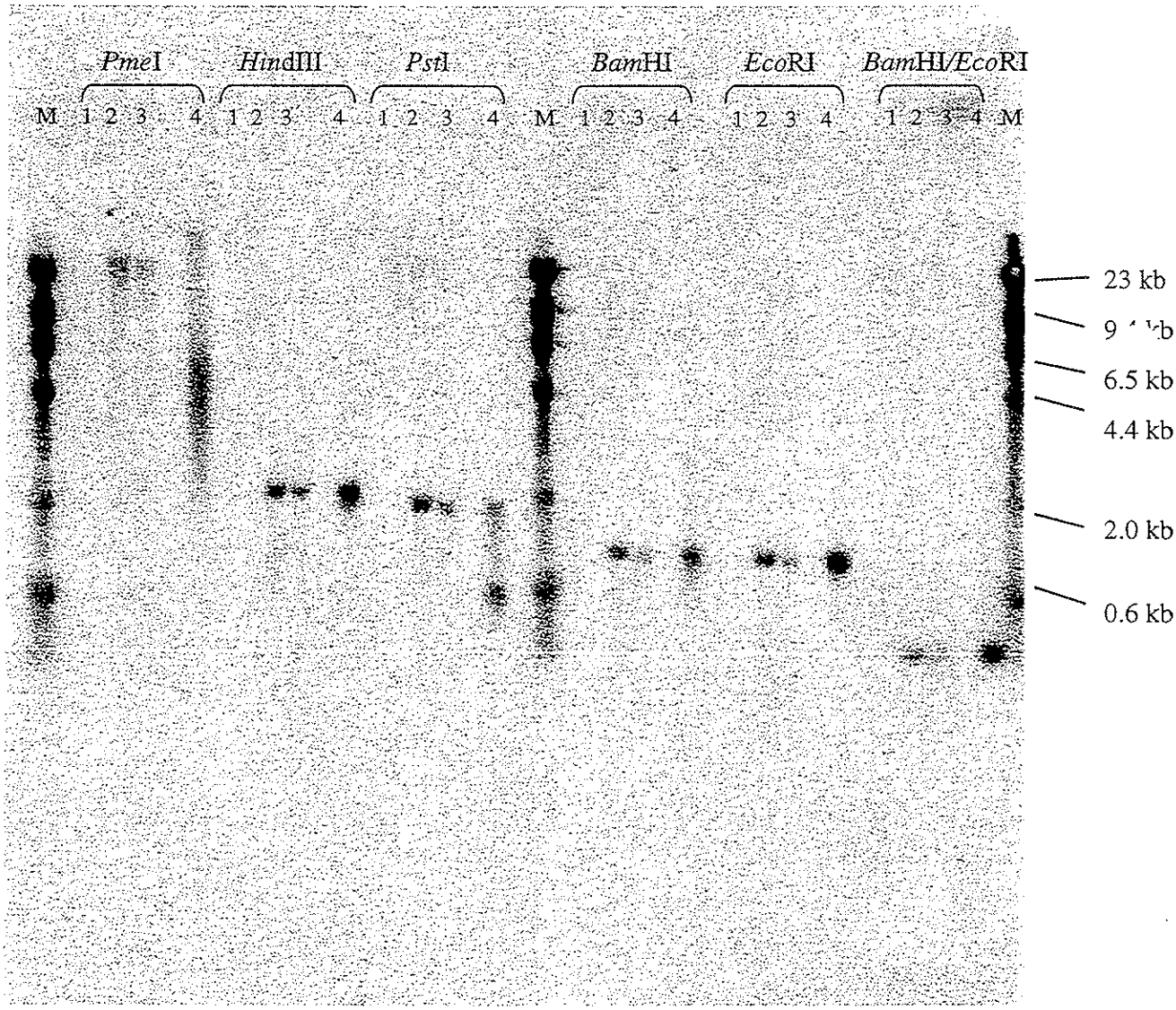
1: Non-transgenic control (5 µg)
2: Pooled DNA from the T1S1 generation (5 µg)
3: Pooled DNA from the BC4 generation (5 µg)
4: Plasmid PHP8999 control (9.5 pg, equivalent to 1 copy)
M: Molecular weight markers from *Hin*dIII digest of lambda DNA (1 µg)

Figure 9: Southern blot analysis of the *cry*1F gene in the DNA insert of 1507 maize



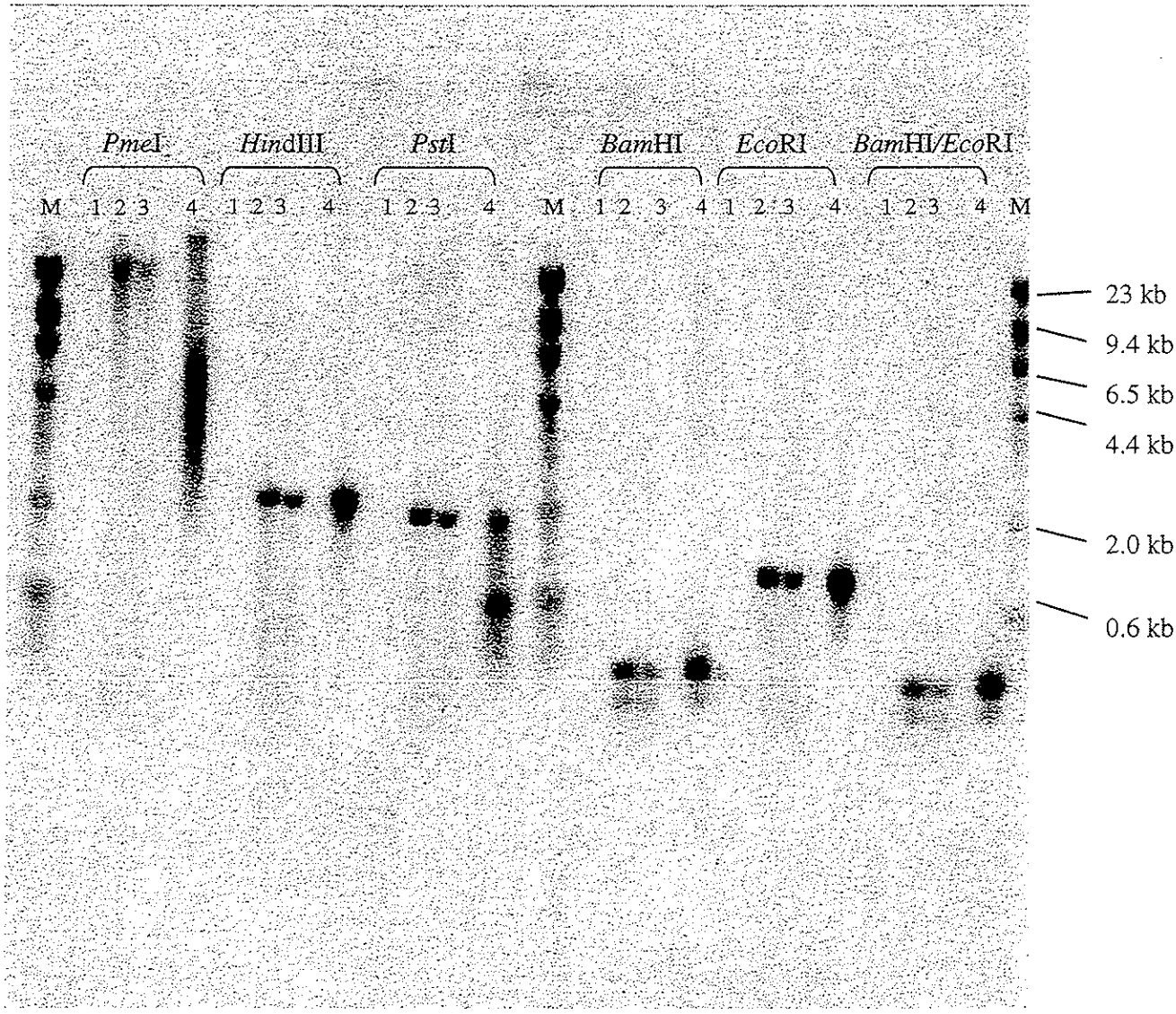
1: Non-transgenic control (5 µg)
2: Pooled DNA from the T1S1 generation (5 µg)
3: Pooled DNA from the BC4 generation (5 µg)
4: Plasmid PHP8999 control (9.5 pg, equivalent to 1 copy)
M: Molecular weight markers from *Hin*dIII digest of lambda DNA (1 µg)

Figure 10: Southern blot analysis of the CaMV 35S promoter for the *pat* gene in the DNA insert of 1507 maize



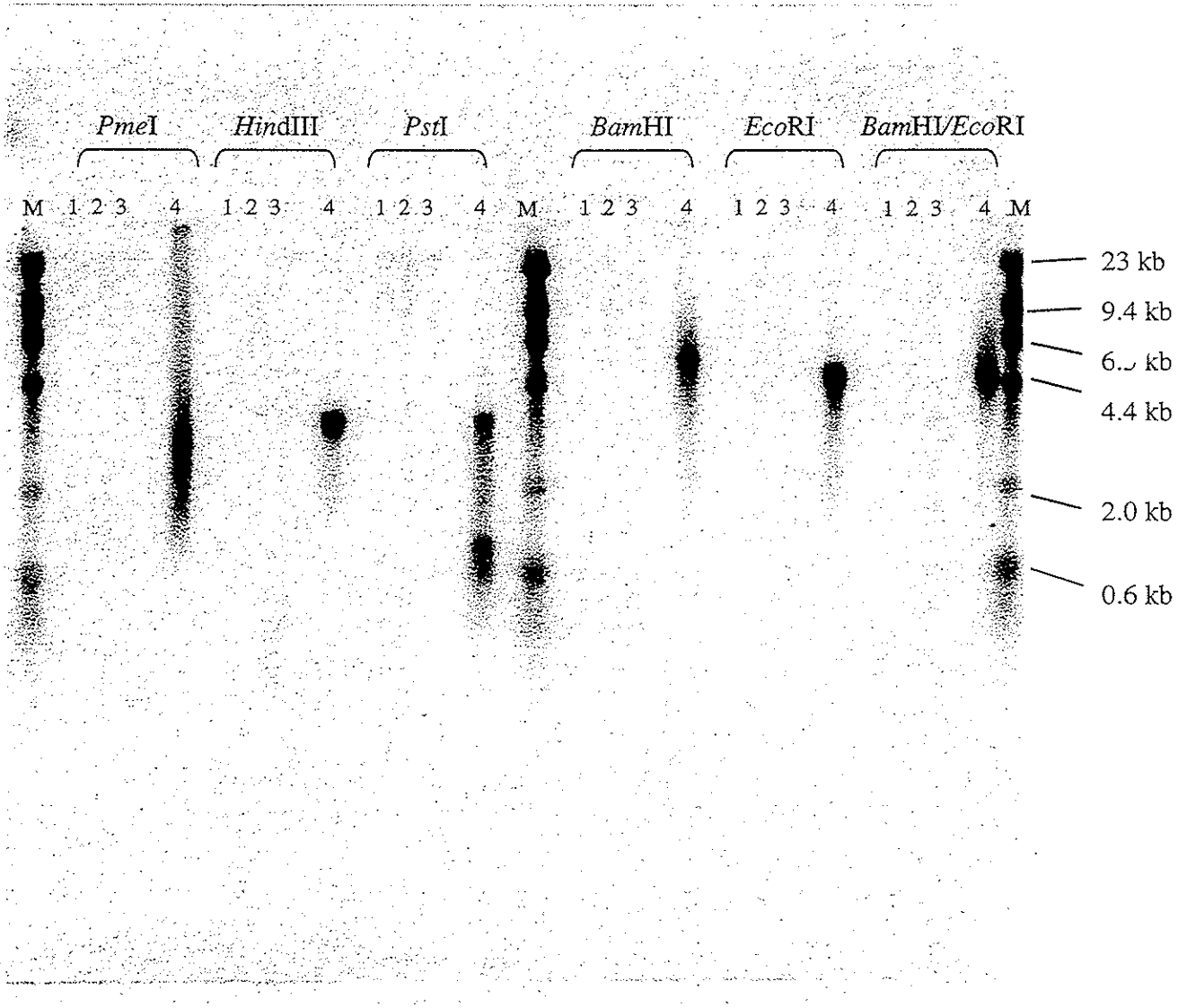
1: Non-transgenic control (5 µg)
2: Pooled DNA from the T1S1 generation (5 µg)
3: Pooled DNA from the BC4 generation (5 µg)
4: Plasmid PHP8999 control (9.5 pg, equivalent to 1 copy)
M: Molecular weight markers from *Hin*dIII digest of lambda DNA (1 µg)

Figure 11: Southern blot analysis of the *pat* gene in the DNA insert of 1507 maize



1: Non-transgenic control (5 µg)
2: Pooled DNA from the T1S1 generation (5 µg)
3: Pooled DNA from the BC4 generation (5 µg)
4: Plasmid PHP8999 control (9.5 pg, equivalent to 1 copy)
M: Molecular weight markers from *Hin*dIII digest of lambda DNA (1 µg)

Figure 12: Southern blot analysis confirming the absence of the *nptII* gene in the DNA insert of 1507 maize



1: Non-transgenic control (5 µg)
2: Pooled DNA from the T1S1 generation (5 µg)
3: Pooled DNA from the BC4 generation (5 µg)
4: Plasmid PHP8999 control (9.5 pg, equivalent to 1 copy)
M: Molecular weight markers from *Hin*dIII digest of lambda DNA (1 µg)

Figure 13: Restriction map showing the exact locations on insert PHI8999A of the *Hin*dIII probes used for the molecular characterization of the genetic material in 1507 maize. The numbers refer to the location of the restriction sites on plasmid PHP8999

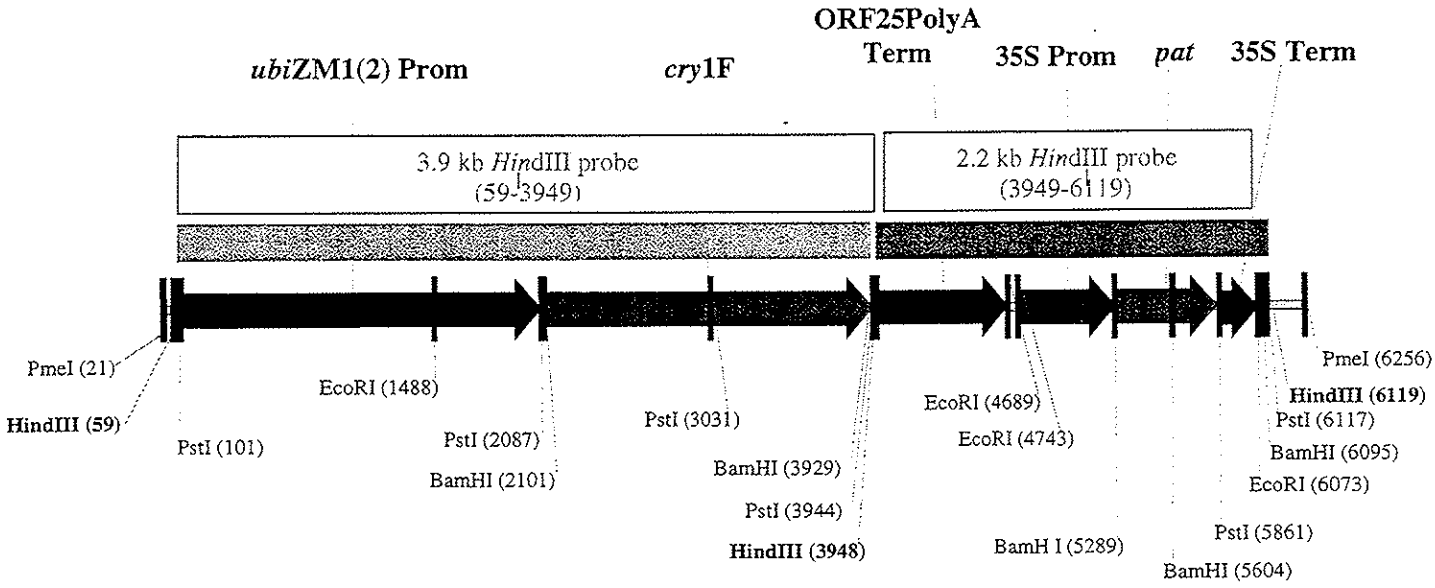
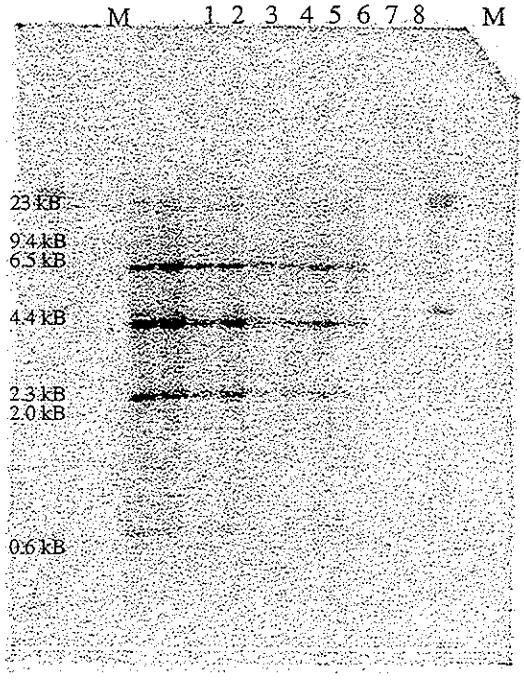
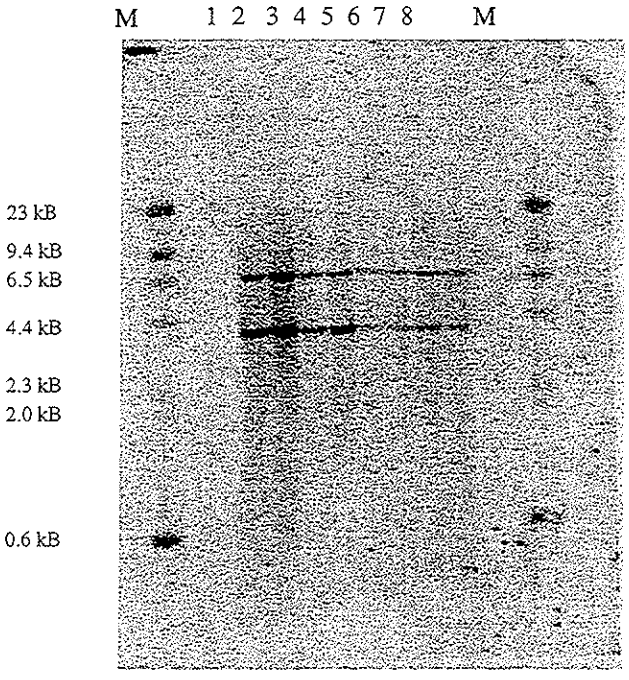


Figure 14: Southern blot analysis of 1507 maize genomic DNA samples digested with *Hin*dIII restriction enzyme and probed with *i)* whole-length plasmid PHP8999; *ii)* 3.9 kb *Hin*dIII fragment corresponding to insert PHI8999A; and, *iii)* 2.2 kb *Hin*dIII fragment corresponding to insert PHI8999A. Samples are loaded from left to right and correspond to different plants from the T1 generation (Lanes 1 to 4), and from the BC4 generation (Lanes 5 to 8). The molecular weight markers (M) is a standard *Hin*dIII digest of lambda DNA

***i)* PROBE = whole-length PHP8999**



***ii)* PROBE = 3.9 kb**



***iii)* PROBE = 2.2 kb**

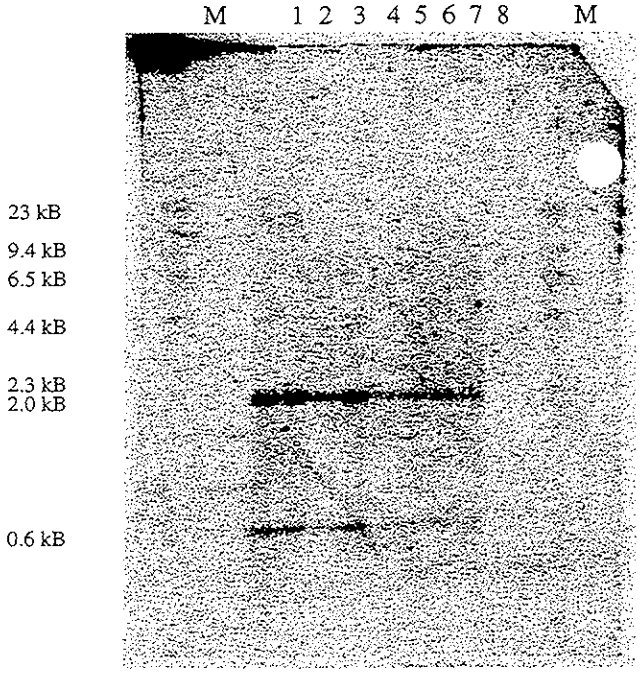
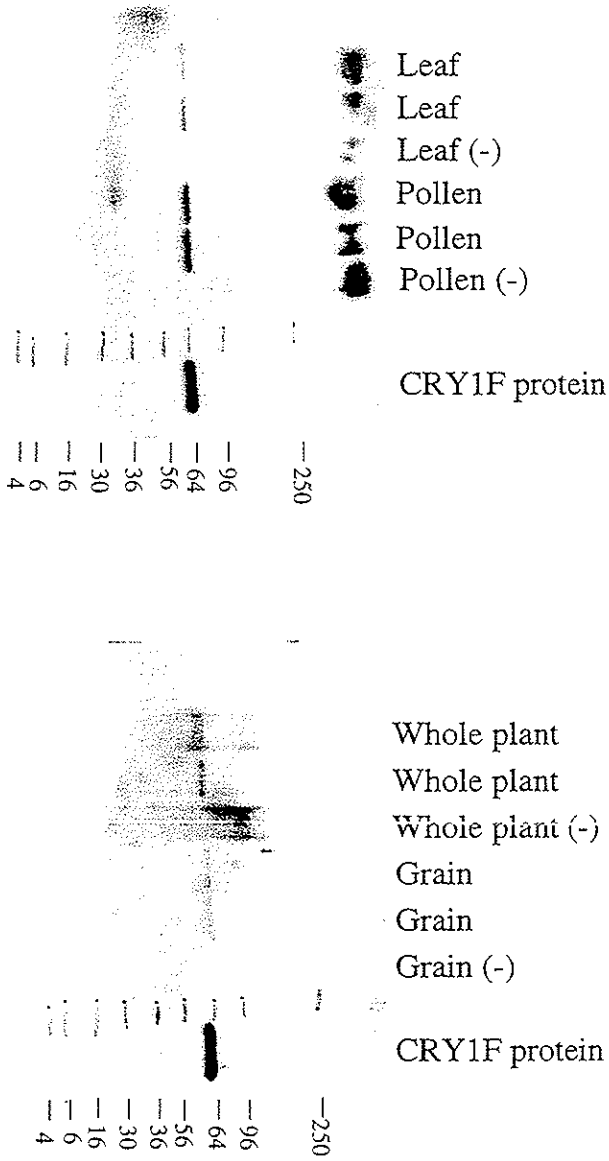


Figure 15: Immunoreactivity of the CRY1F protein expressed in tissues of 1507 maize



Lanes are labeled as follows: Leaf: Leaf tissue from two individual 1507 maize plants (23 µg and 55 µg of protein, respectively). Leaf (-): Leaf tissue from non-GM control maize (41 µg of protein). Pollen: Pollen tissue from two individual 1507 maize plants (41 µg and 61 µg of protein, respectively). Pollen (-): Pollen tissue from non-GM control maize (26 µg of protein). Whole plant: Whole plant tissue from two individual 1507 maize plants (12 µg and 6 µg of protein, respectively). Whole plant (-): Whole plant tissue from non-GM control maize (14 µg of protein). Grain: Grain tissue from two individual 1507 maize plants (82 µg and 98 µg of protein, respectively). Grain (-): Grain tissue from non-GM control maize (79 µg of protein). CRY1F protein: Purified microbially-derived CRY1F protein. Molecular weight standards from 4 kilodaltons (kD) to 250 kD are indicated on the figure. Electrophoresis was conducted under denaturing conditions.

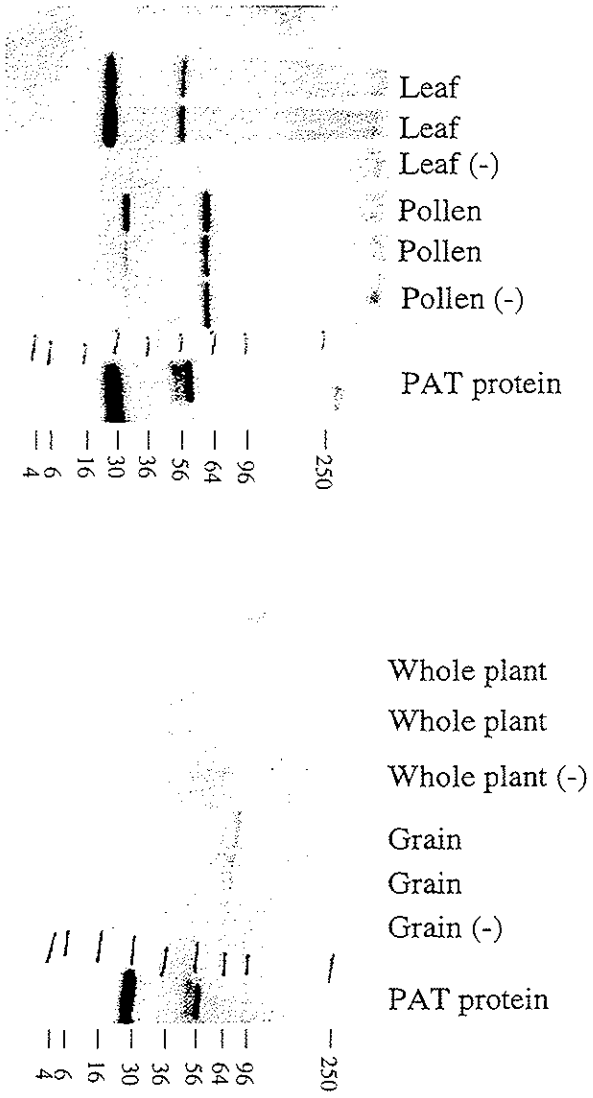
Figure 16: Amino acid sequence of CRY1F protein expressed in 1507 maize and molecular weight prediction using the ExPASy Server. The position of amino acid ^{27}R is shown in bold and that of ^{604}L in italics.

^{1}MENNIQNQCVPYNCLNNPEVEILNEE**R**STGRLPLDISLSLTRFLLSEFVPGVG
VAFGLFDLIWGFITPSDWSLFLLQIEQLIEQRIETLERNRAITTLRGLADSYEIYI
EALREWEANPNNAQLREDVRIRFANTDDALITAINNFTLTSFEIPLLSVYVQAA
NLHLSLLRDAVSFGQGWGLDIATVNNHYNRLINLIHRYTKHCLDTYNQGLEN
LRGTNTRQWARFNQFRRDLTLTVLDIVALFPNYDVRTYPIQTSSQLTREIYTSS
VIEDSPVSANIPNGFNRAEFGVRPPHLMDFMNSLFVTAETVRSQTVWGGHLV
SSRNTAGNRINFPSYGVFNPGGAIWIADEDPRPFYRTLSDPVFVRGGFGNPHY
VLGLRGVAFQQTGTNHTRTFRNSGTIDSLDEIPPQDNSGAPWNDYSHVLNHV
TFVRWPGEISGSDSWRAPMFSWTHRSATPTNTIDPERITQIPLVKAHTLQSGTT
VVRGPGFTGGDILRRTSGGPFAYTIVNINGQLPQRYRARIRYASTTNLRIYVTV
AGERIFAGQFNKTMDTGDPLTFQSFSYATINTAFTFPMSQSSFTVGADTFSSGN
EVYIDRFELIPVTAT*L*E^{605}

Theoretical molecular weight: 68204.56 Da

The theoretical molecular weight of the CRY1F sequence ^{28}STGR... ... AT*L*E^{605} is 65002.03 Da, which corresponds to the 65 kDa form also obtained from maize, as discussed in the text.

Figure 17: Immunoreactivity of the PAT protein expressed in tissues of 1507 maize



Lanes are labeled as follows: Leaf: Leaf tissue from two individual 1507 maize plants (23 µg and 55 µg of protein, respectively). Leaf (-): Leaf tissue from non-GM control maize (41 µg of protein). Pollen: Pollen tissue from two individual 1507 maize plants (41 µg and 61 µg of protein, respectively). Pollen (-): Pollen tissue from non-GM control maize (26 µg of protein). Whole plant: Whole plant tissue from two individual 1507 maize plants (12 µg and 6 µg of protein, respectively). Whole plant (-): Whole plant tissue from non-GM control maize (14 µg of protein). Grain: Grain tissue from two individual 1507 maize plants (82 µg and 98 µg of protein, respectively). Grain (-): Grain tissue from non-GM control maize (79 µg of protein). PAT protein: Purified PAT protein. Molecular weight standards from 4 kilodaltons (kD) to 250 kD are indicated on the figure. Electrophoresis was conducted under denaturing conditions.

Figure 18: Backcrossing to integrate desired genes from a "donor" plant (GM) into germplasm of an elite line (non-GM elite inbred). The insert and the two genomes are represented by different colours: i) The red colour indicates the inserted genes; ii) the blue colour represents the percentage of genetic material from the donor; and, iii) the yellow colour corresponds to the percentage of genetic material that is identical to the elite inbred also known as recurrent parent (RP). The plants that do not carry the inserted genes are not selected. The resulting hybrid is then selected and selfed to obtain the elite inbred homozygous for the desired genes

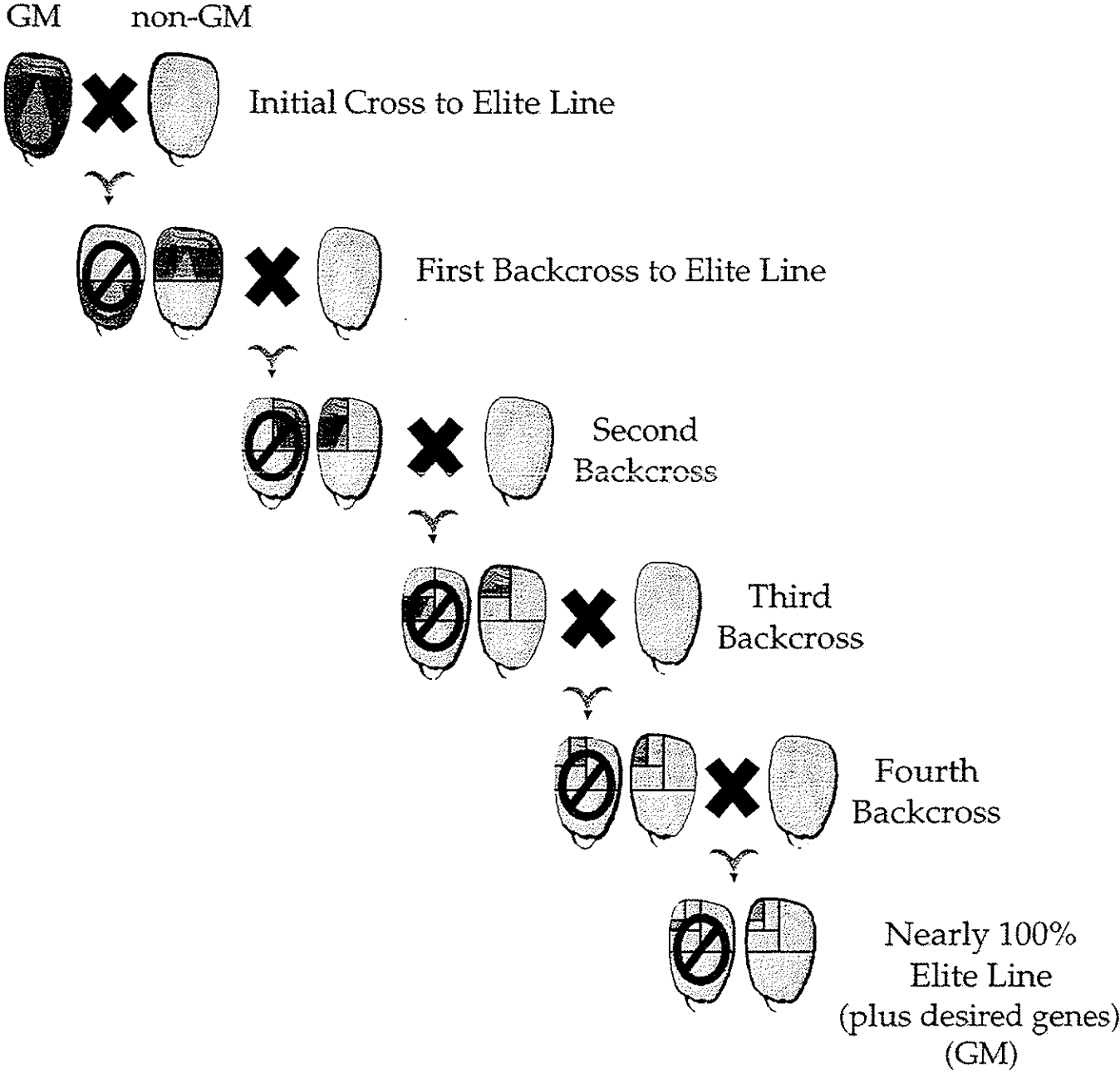


Figure 19: Backcrossing generations used to determine Mendelian segregation ratios for 1507 maize

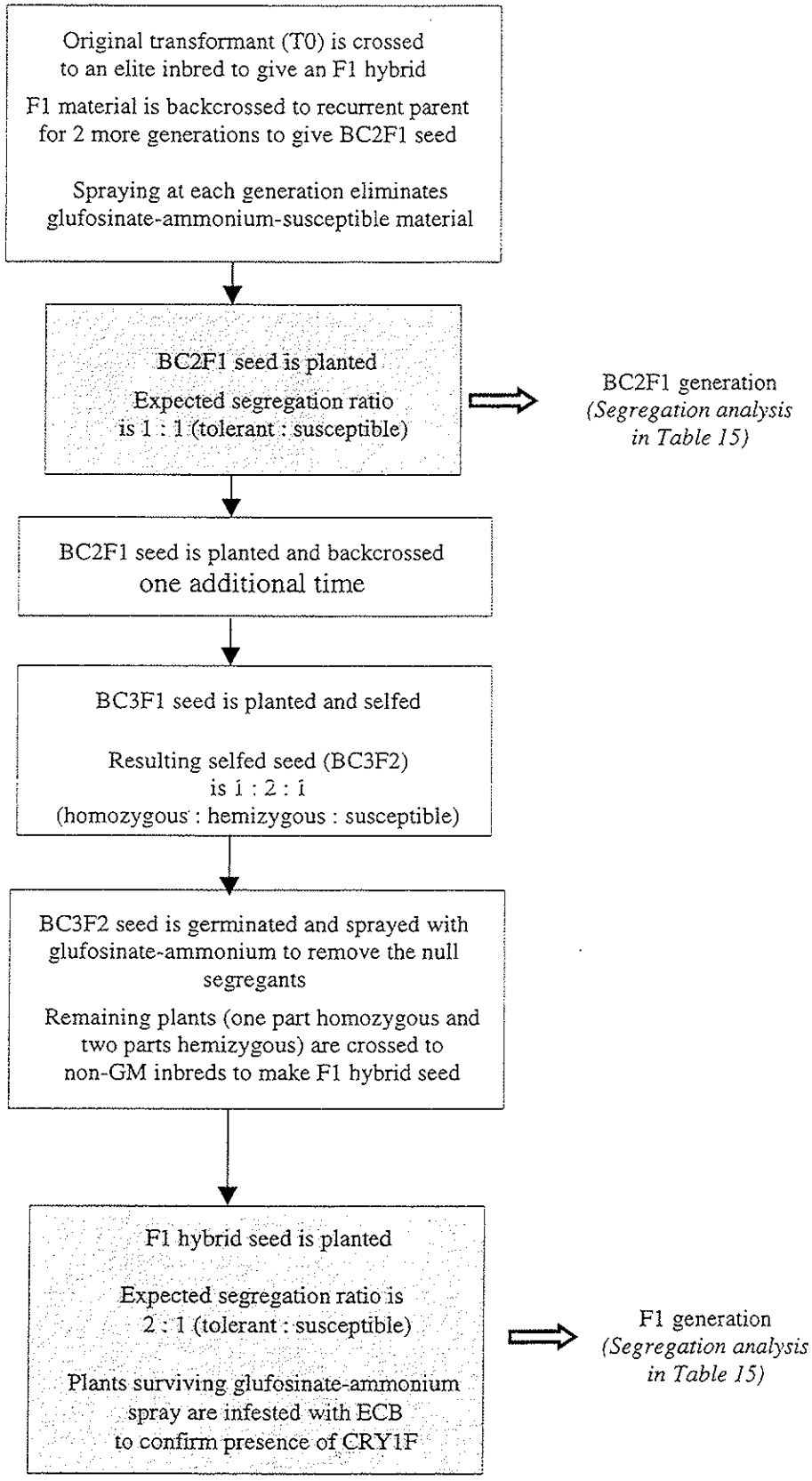


Figure 20: Diagram of the 1507 maize insert

The diagram of the 1507 maize insert is divided into three separate major sections as referred to in the text; the 5' region that includes the border with maize genomic DNA and other DNA segments as described in Table 18, the full-length insert of PHI8999A fragment in the center of the diagram, and the 3' region that includes the inverted ORF25 terminator and additional sequence as described in the Table 18. Arrows beneath the diagram of the insert indicate the two novel open reading frames, ORF3 and ORF4, and other maize endogenous open reading frames identified. Numbers next to DNA segment names refer to the region numbers as described in Table 18.

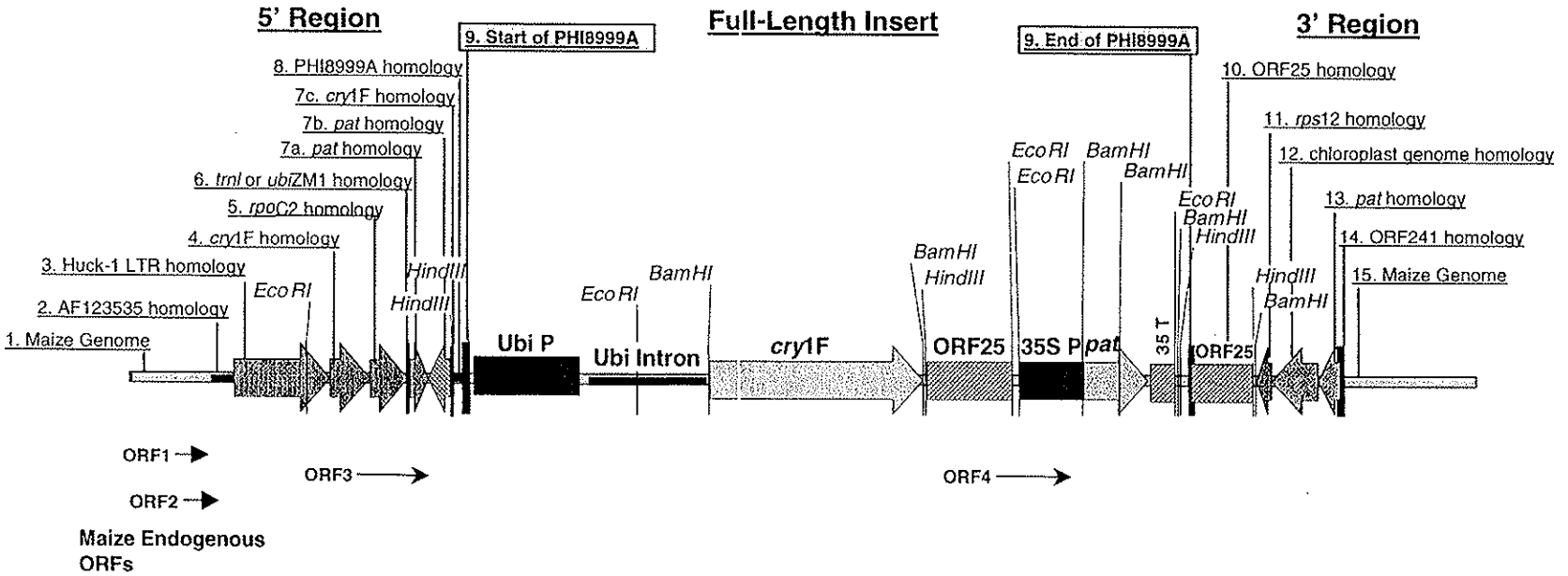


Figure 21: PCR analysis on genomic DNA for sequences 5' to the full-length insert in 1507 maize and for two regions unique to the 1507 maize insert

Hi-II maize was used as the non-GM control. The expected amplicon size is indicated above the lanes and the regions refer to those listed in Table 3. Genomic DNA samples prepared from two different plants of 1507 maize are designated as 1507-1 and 1507-2

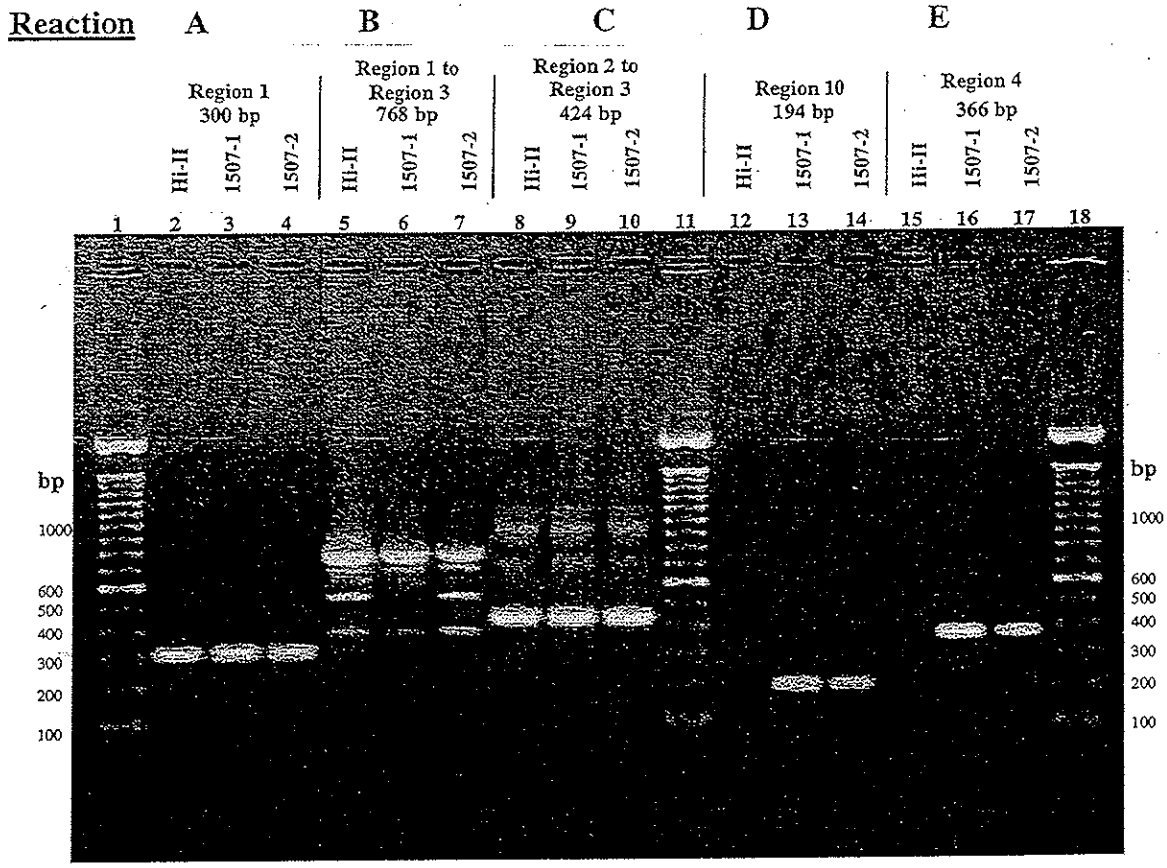


Figure 22: PCR analysis on genomic DNA for regions 13 to 15 at the 1507 maize insert 3' border sequence

The expected amplicon size is indicated above the lanes and the regions refer to those listed in Table 1. Genomic DNA samples prepared from two different plants of 1507 maize are designated as 1507-1 and 1507-2

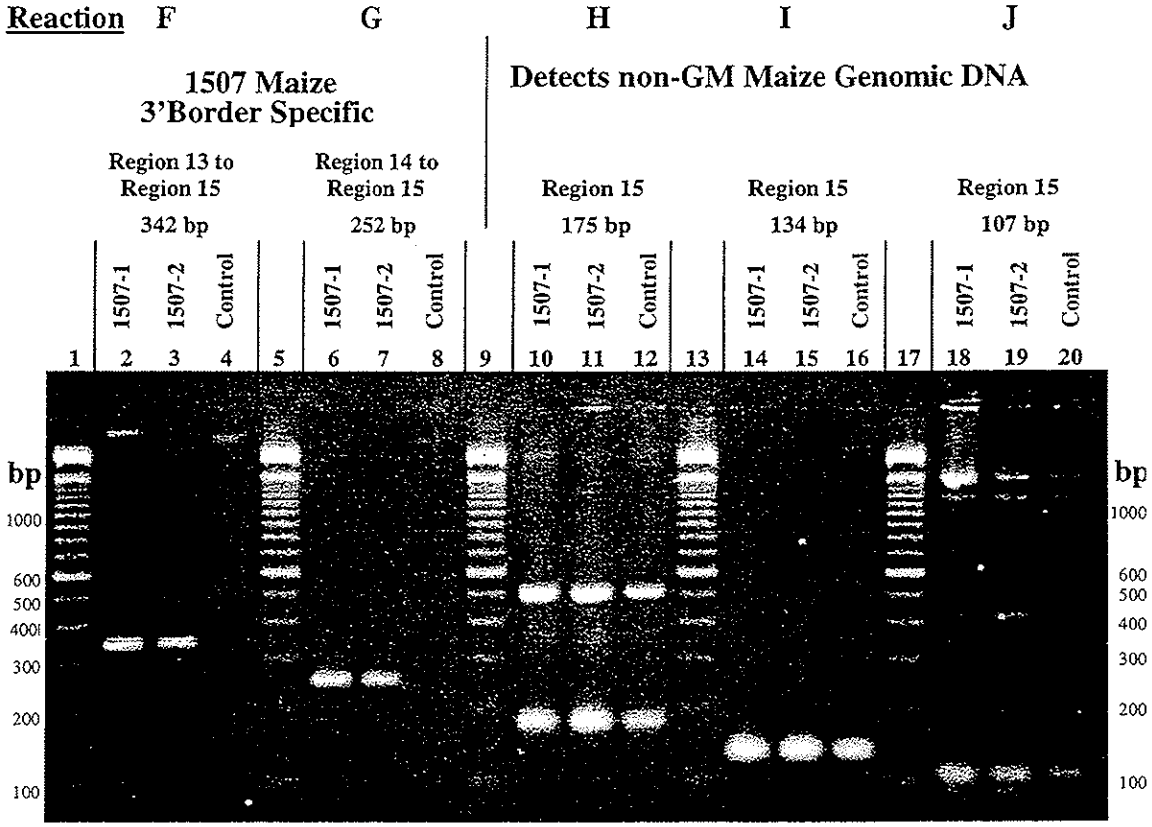


Figure 23: Plasmid map of PHP8999 with probe locations indicated

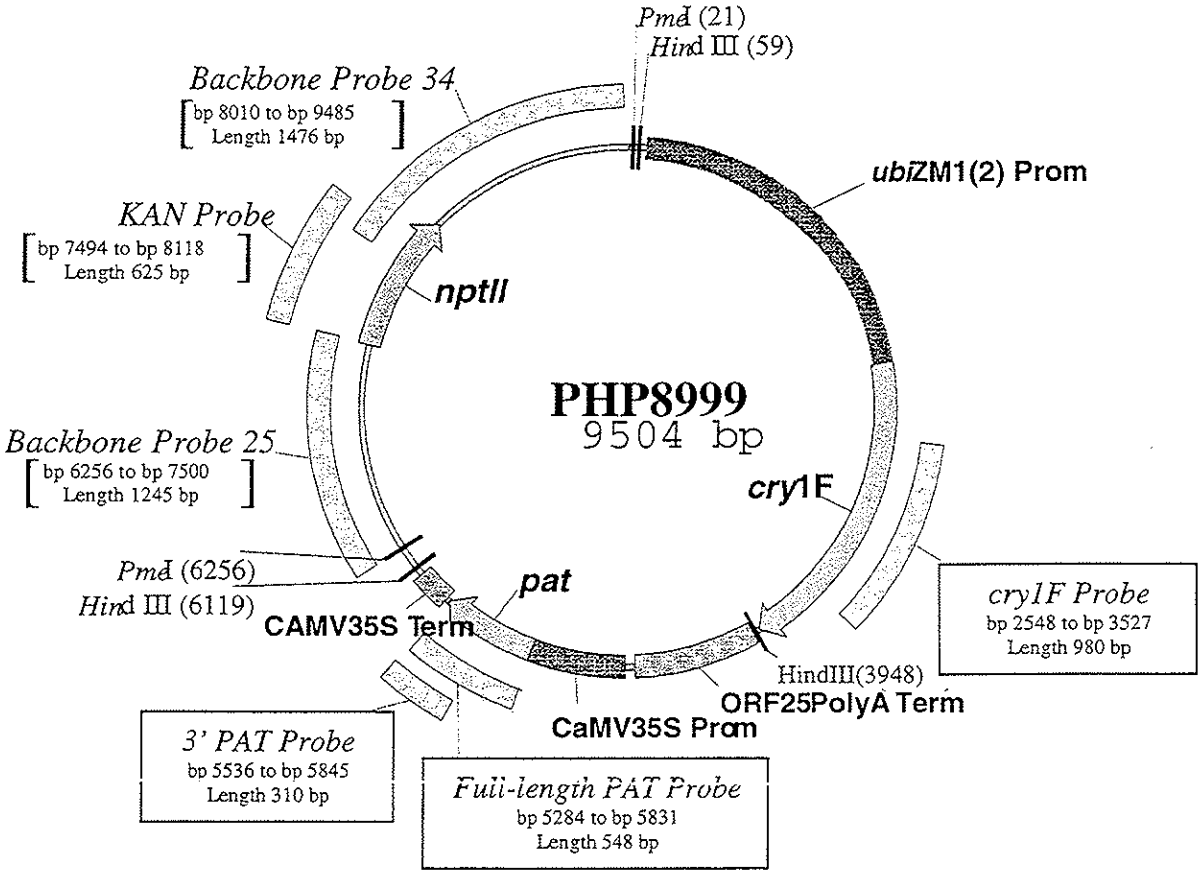
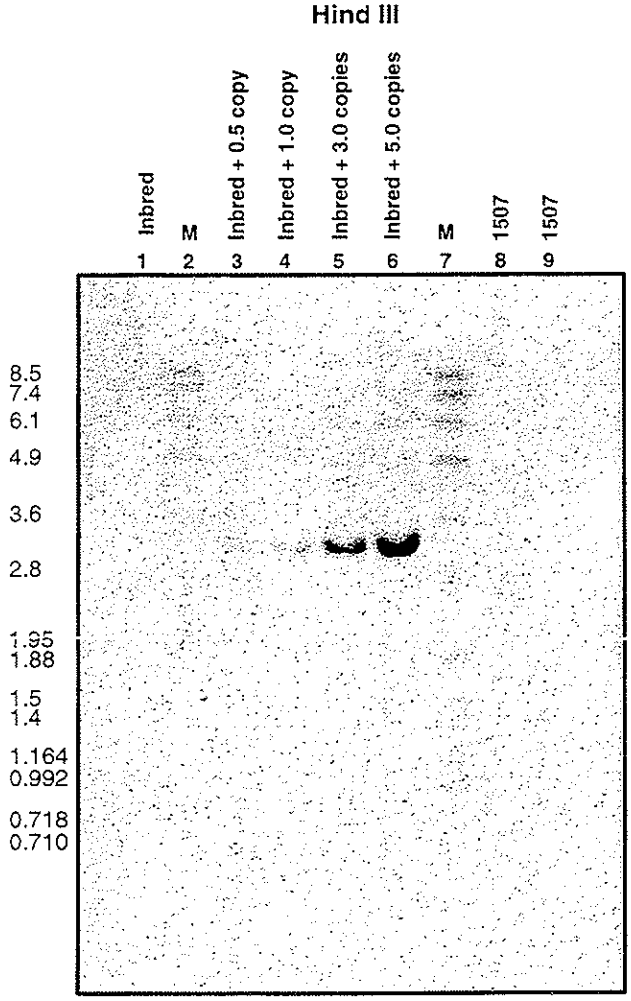


Figure 24: Southern blot analysis of 1507 maize insert with the *kan* probe

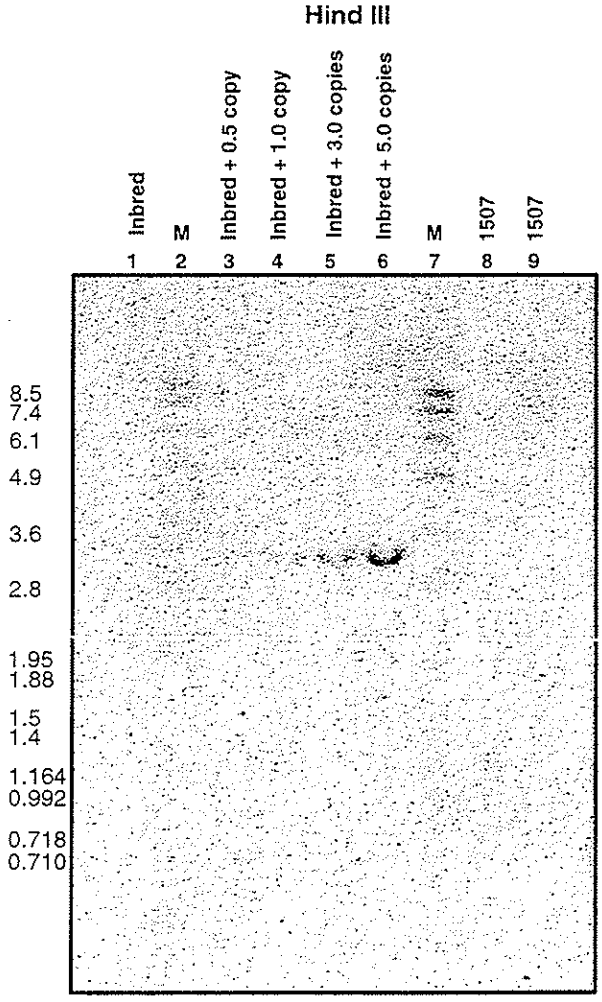
DNA isolated from 1507 maize and non-GM control inbred maize was digested with *Hind* III and probed with the *kan* probe. Approximately 5 µg of genomic DNA was digested and loaded per lane. The gene copy number controls included plasmid PHP8999 at the indicated approximate gene copy number equivalents and 5 µg of non-GM control inbred maize genomic DNA.



Gel Loading:
Lane 1 – Inbred *Hind* III
Lane 2 – Dig VII Marker (Roche)
Lane 3 – Inbred + 0.5 copy PHP8999 *Hind* III
Lane 4 – Inbred + 1.0 copy PHP8999 *Hind* III
Lane 5 – Inbred + 3.0 copies PHP8999 *Hind* III
Lane 6 – Inbred + 5.0 copies PHP8999 *Hind* III
Lane 7 – Dig VII Marker (Roche)
Lane 8 – TC1507 *Hind* III
Lane 9 – TC1507 *Hind* III

Figure 25: Southern blot analysis of 1507 maize insert with Backbone Probe 25

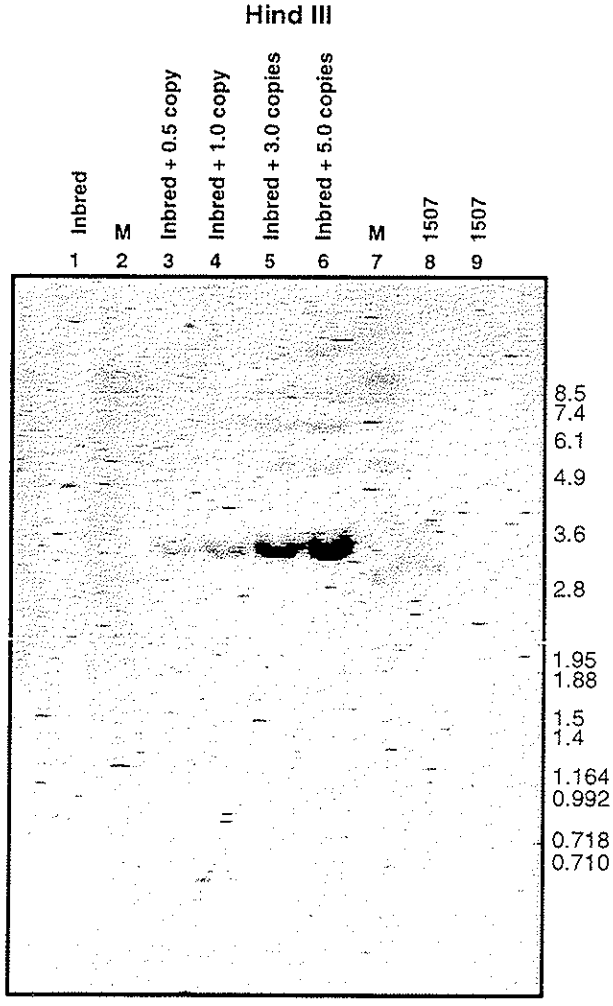
DNA isolated from 1507 maize and non-GM control inbred maize was digested with *Hind* III and probed with Backbone Probe 25. Approximately 5 µg of genomic DNA was digested and loaded per lane. The gene copy number controls included plasmid PHP8999 at the indicated approximate gene copy number equivalents and 5 µg of non-GM control inbred maize genomic DNA.



Gel Loading:
Lane 1 – Inbred *Hind* III
Lane 2 – Dig VII Marker (Roche)
Lane 3 – Inbred + 0.5 copy PHP8999 *Hind* III
Lane 4 – Inbred + 1.0 copy PHP8999 *Hind* III
Lane 5 – Inbred + 3.0 copies PHP8999 *Hind* III
Lane 6 – Inbred + 5.0 copies PHP8999 *Hind* III
Lane 7 – Dig VII Marker (Roche)
Lane 8 – TC1507 *Hind* III
Lane 9 – TC1507 *Hind* III

Figure 26: Southern blot analysis of 1507 maize insert with Backbone Probe 34

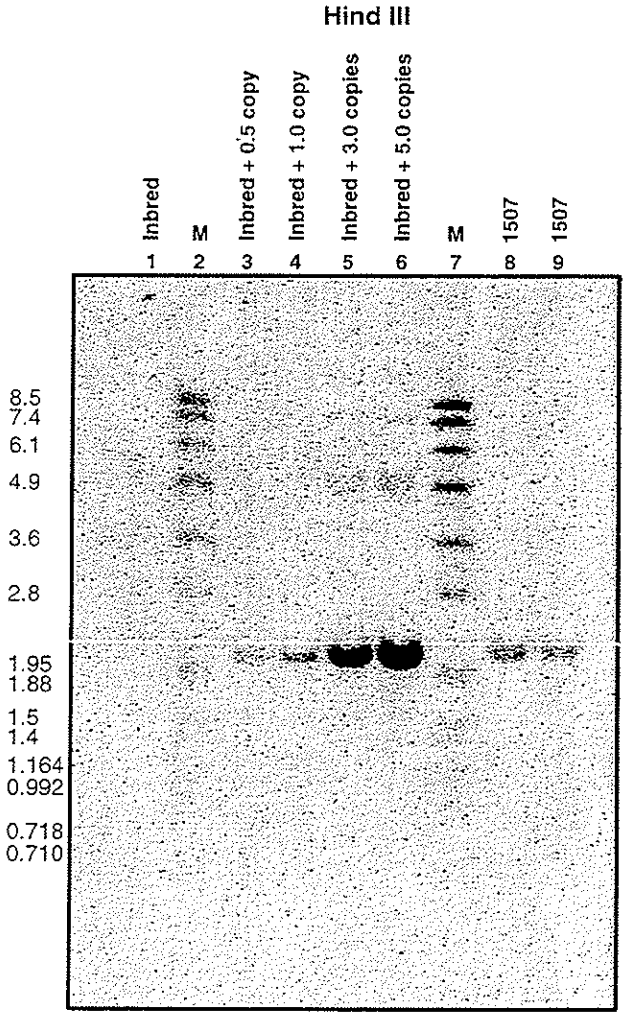
DNA isolated from 1507 maize and non-GM control inbred maize was digested with *Hind* III and probed with Backbone Probe 34. Approximately 5 µg of genomic DNA was digested and loaded per lane. The gene copy number controls included plasmid PHP8999 at the indicated approximate gene copy number equivalents and 5 µg of non-GM control inbred maize genomic DNA.



Gel Loading:
Lane 1 – Inbred *Hind* III
Lane 2 – Dig VII Marker (Roche)
Lane 3 – Inbred + 0.5 copy PHP8999 *Hind* III
Lane 4 – Inbred + 1.0 copy PHP8999 *Hind* III
Lane 5 – Inbred + 3.0 copies PHP8999 *Hind* III
Lane 6 – Inbred + 5.0 copies PHP8999 *Hind* III
Lane 7 – Dig VII Marker (Roche)
Lane 8 – TC1507 *Hind* III
Lane 9 – TC1507 *Hind* III

Figure 27: Southern blot analysis of 1507 maize insert with the full-length *pat* probe

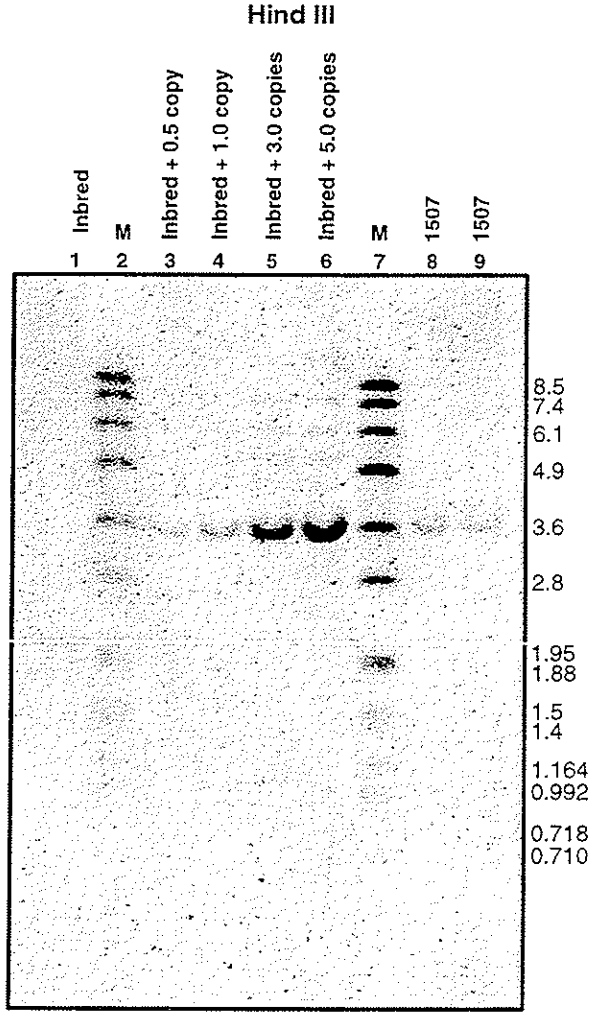
DNA isolated from 1507 maize and non-GM control inbred maize was digested with *Hind* III and probed with the full-length *pat* probe. Approximately 5 µg of genomic DNA was digested and loaded per lane. The gene copy number controls included plasmid PHP8999 at the indicated approximate gene copy number equivalents and 5 µg of non-GM control inbred maize genomic DNA.



Gel Loading:
Lane 1 – Inbred *Hind* III
Lane 2 – Dig VII Marker (Roche)
Lane 3 – Inbred + 0.5 copy PHP8999 *Hind* III
Lane 4 – Inbred + 1.0 copy PHP8999 *Hind* III
Lane 5 – Inbred + 3.0 copies PHP8999 *Hind* III
Lane 6 – Inbred + 5.0 copies PHP8999 *Hind* III
Lane 7 – Dig VII Marker (Roche)
Lane 8 – TC1507 *Hind* III
Lane 9 – TC1507 *Hind* III

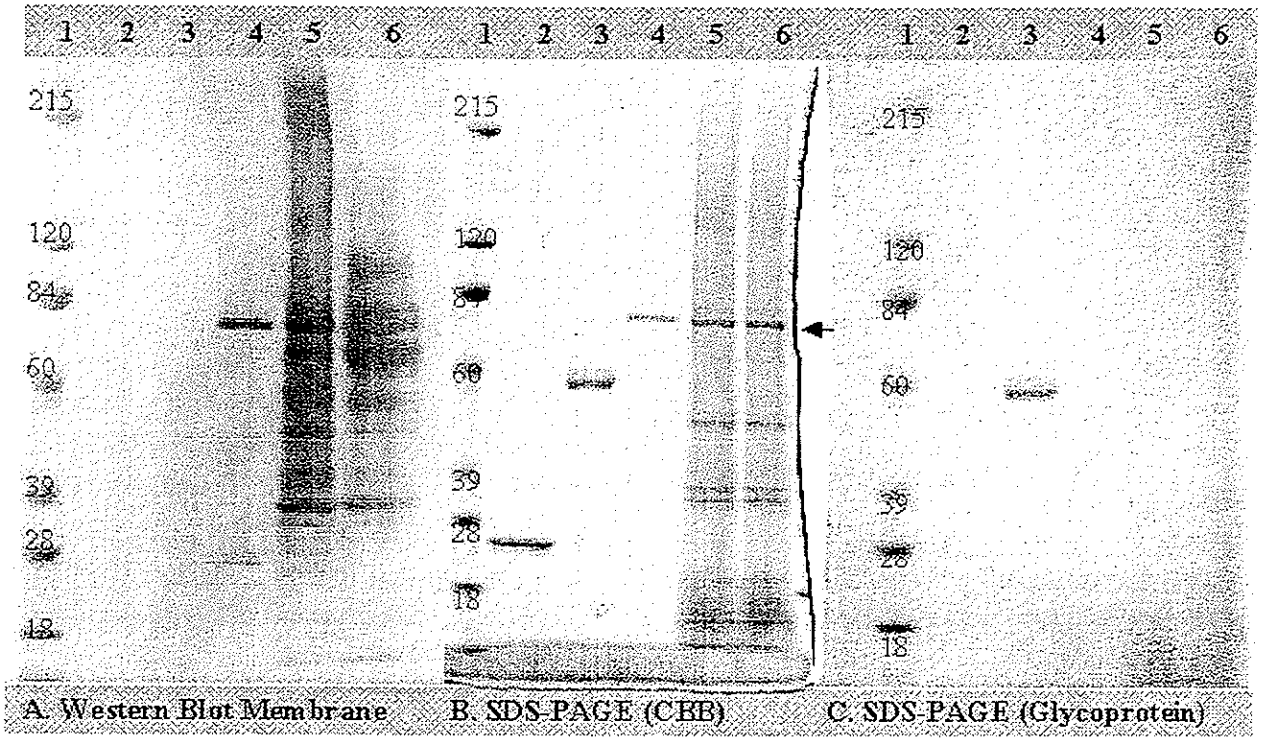
Figure 28: Southern blot analysis of 1507 maize insert with the *cry*1F probe

DNA isolated from 1507 maize and non-GM control inbred maize was digested with *Hind* III and probed with the *cry*1F probe. Approximately 5 µg of genomic DNA was digested and loaded per lane. The gene copy number controls included plasmid PHP8999 at the indicated approximate gene copy number equivalents and 5 µg of non-GM control inbred maize genomic DNA.



Gel Loading:
Lane 1 – Inbred *Hind* III
Lane 2 – Dig VII Marker (Roche)
Lane 3 – Inbred + 0.5 copy PHP8999 *Hind* III
Lane 4 – Inbred + 1.0 copy PHP8999 *Hind* III
Lane 5 – Inbred + 3.0 copies PHP8999 *Hind* III
Lane 6 – Inbred + 5.0 copies PHP8999 *Hind* III
Lane 7 – Dig VII Marker (Roche)
Lane 8 – TC1507 *Hind* III
Lane 9 – TC1507 *Hind* III

Figure 29: Analysis of the lack of post-translational glycosylation of microbially-derived and maize expressed CRY1F proteins (Annex 17). Arrow denotes CRY1F protein. The glycoprotein horseradish peroxidase was used as a positive control and the non-glycosylated protein soybean trypsin inhibitor as a negative control for glycosylation.

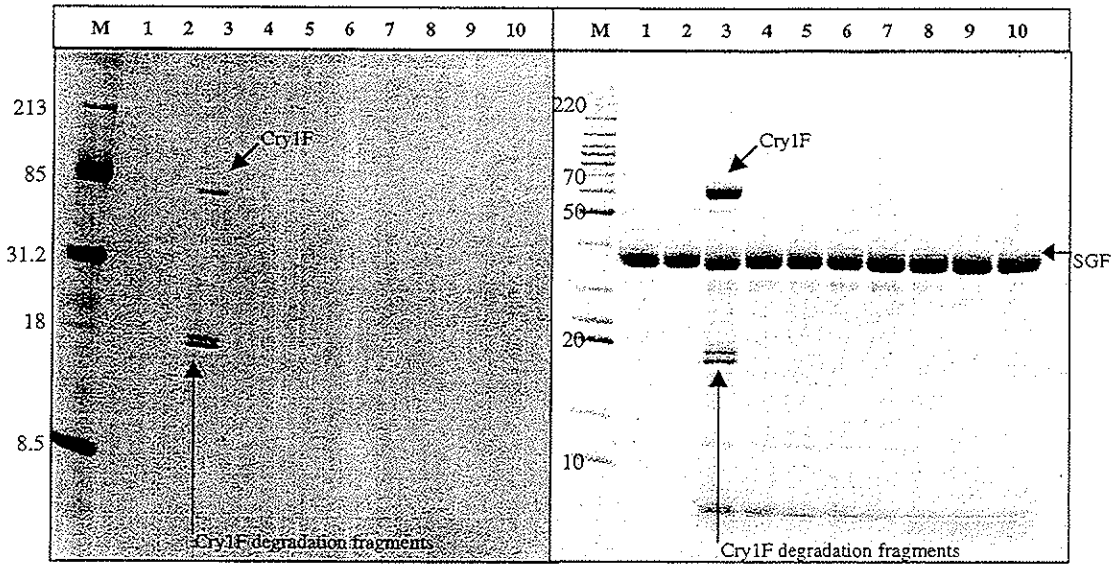


Panel A: Western blot detected with rabbit anti-CRY1F polyclonal antibody
Panel B: SDS-PAGE, 4-20% gradient gel, Coomassie brilliant blue (CBB) stained
Panel C: SDS-PAGE, 4-20% gradient gel, GelCode glycoprotein stained

Lane 1: Pre-stained molecular weight markers (Pierce Chemical, BlueRanger)
Lane 2: Soybean trypsin inhibitor (MW: 20.1 kDa), 1.25 µg/lane
Lane 3: Horseradish peroxidase (MW: 44 kDa), 1.25 µg/lane
Lane 4: Microbially-derived CRY1F protein, 0.14 µg/lane
Lanes 5 and 6: CRY1F protein expressed in 1507 maize

*Arrow denotes CRY1F protein

Figure 30: Western blot and SDS-PAGE analysis of microbially-derived CRY1F protein digestion in Simulated Gastric Fluid



Panel A: Cry1F Western Blot
Lane Assignments
M - Bio-Rad Prestained Molecular Weight Standards
1 - SGF Reagent Blank, 0 minute incubation
2 - SGF Reagent Blank, 15 minute incubation
3 - CRY1F (0.12 µg), 0 minute digestion
4 - 15 second digestion
5 - 30 second digestion
6 - 1 minute digestion
7 - 2 minute digestion
8 - 5 minute digestion
9 - 10 minute digestion
10 - 15 minute digestion

Panel B: Cry1F SDS-PAGE Gel
Lane Assignments
M - Benchmark Protein Ladder Molecular Weight Standards
1 - SGF Reagent Blank, 0 minute incubation
2 - SGF Reagent Blank, 15 minute incubation
3 - CRY1F (0.58 µg), 0 minute digestion
4 - 15 second digestion
5 - 30 second digestion
6 - 1 minute digestion
7 - 2 minute digestion
8 - 5 minute digestion
9 - 10 minute digestion
10 - 15 minute digestion

* Note: MW markers are labeled in kDa.

Figure 31: Comparison of lepidopteran insect pest attack on non-GM maize control (left) and 1507 maize (right) grown in Calatorao (Aragón) in 2002. Note the lodging of the cobs observed for non-GM maize, while upright for 1507 maize.

Figure 32: Detail of stalk tunnelling damage caused by lepidopteran insect pest attack on non-GM maize control hybrid A (left) and 1507 maize hybrid A (right), with comparable genetic backgrounds, grown in Calatorao (Aragón) in 2002

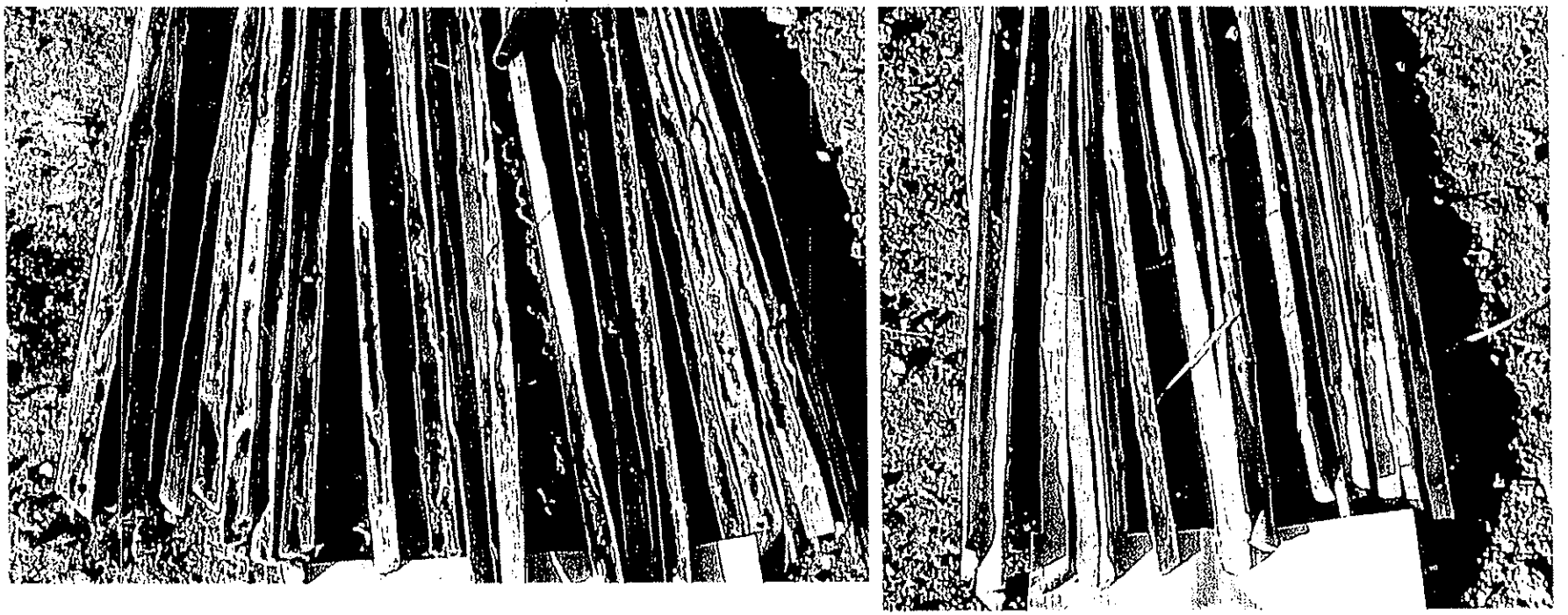


Figure 33: Comparison of lepidopteran insect pest attack on non-GM maize control (left) and 1507 maize (right) grown in Montañana (Aragón) in 2002

Figure 34: Detail of stalk tunnelling damage caused by lepidopteran insect pest attack on non-GM maize control hybrid B (left) and 1507 maize hybrid B (right), with comparable genetic background, grown in Montañana (Aragón) in 2002

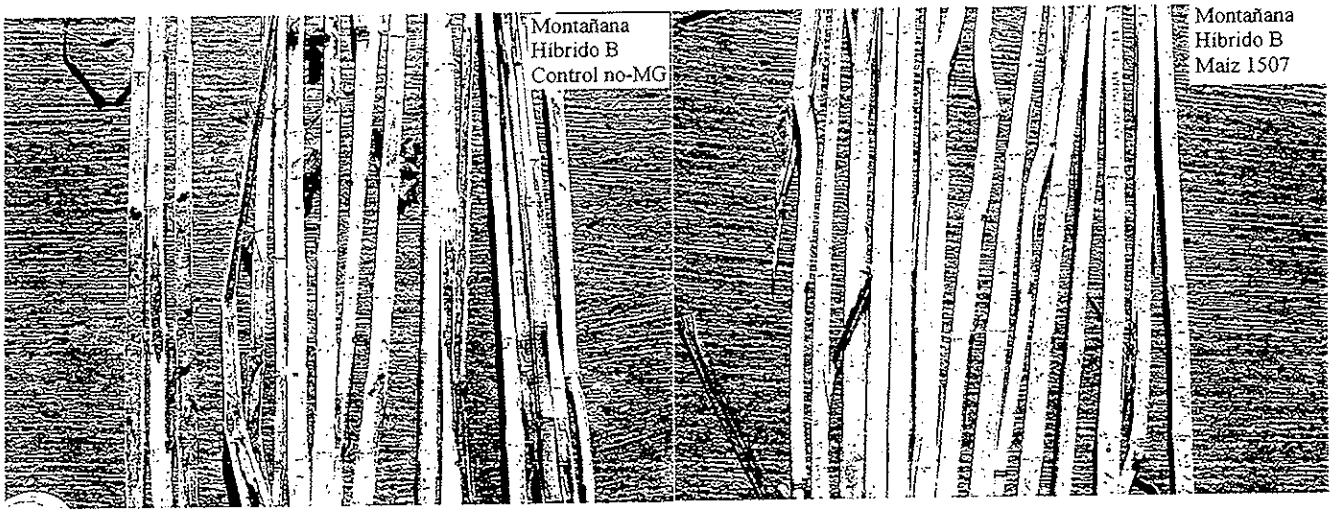


Figure 35: Mean and standard errors for Family Anthocoridae at the Spanish locations of Calatorao and Montañana

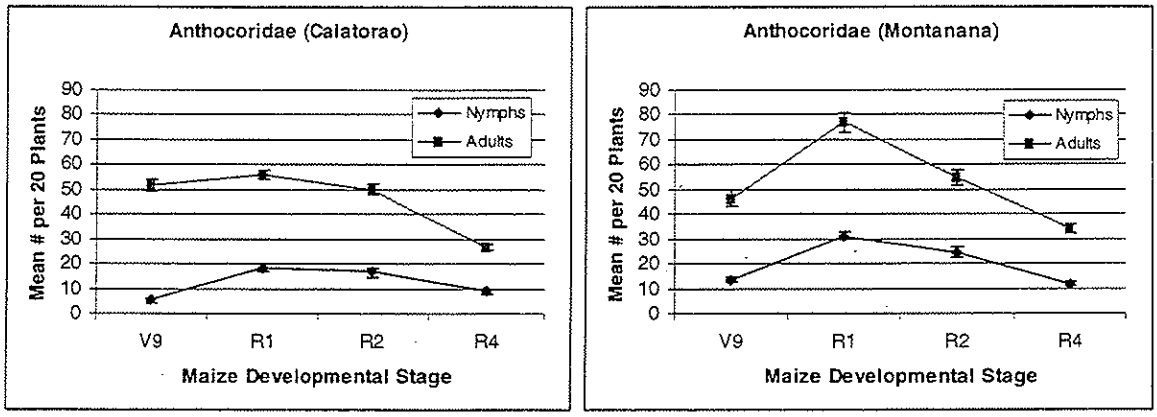


Figure 36: Mean and standard errors for pooled observations on spiders at the Spanish locations of Calatorao and Montañana

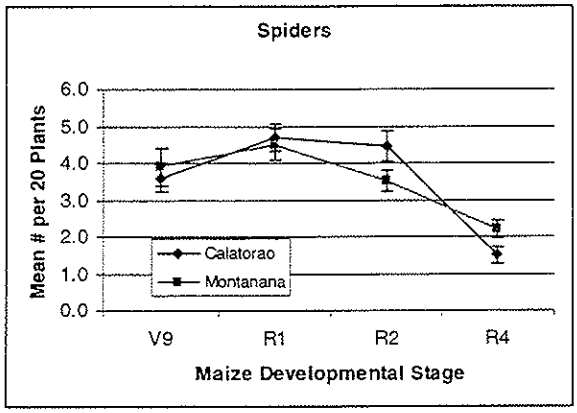


Figure 37: Mean and standard errors for Family Nabidae at both locations at the Spanish locations of Calatorao and Montañana

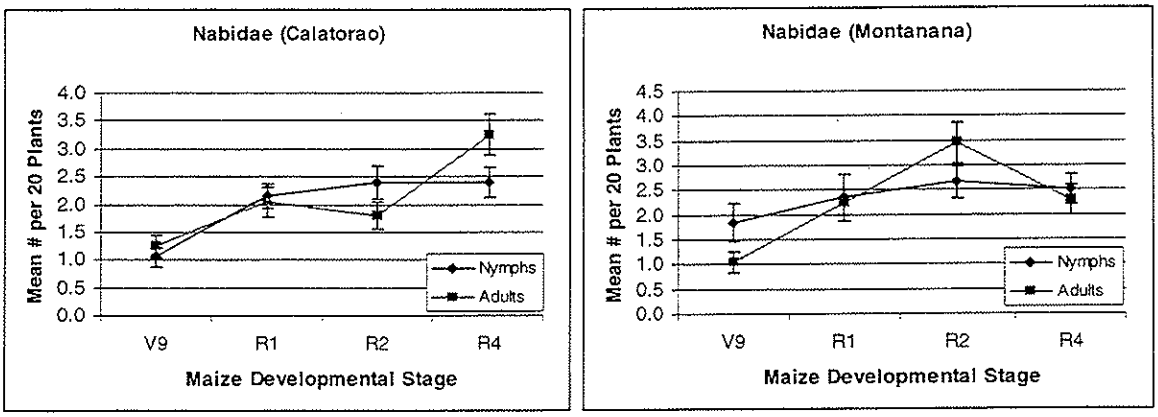


Figure 38: Mean and standard errors for Family Chrysopidae at the Spanish locations of Calatorao and Montañana

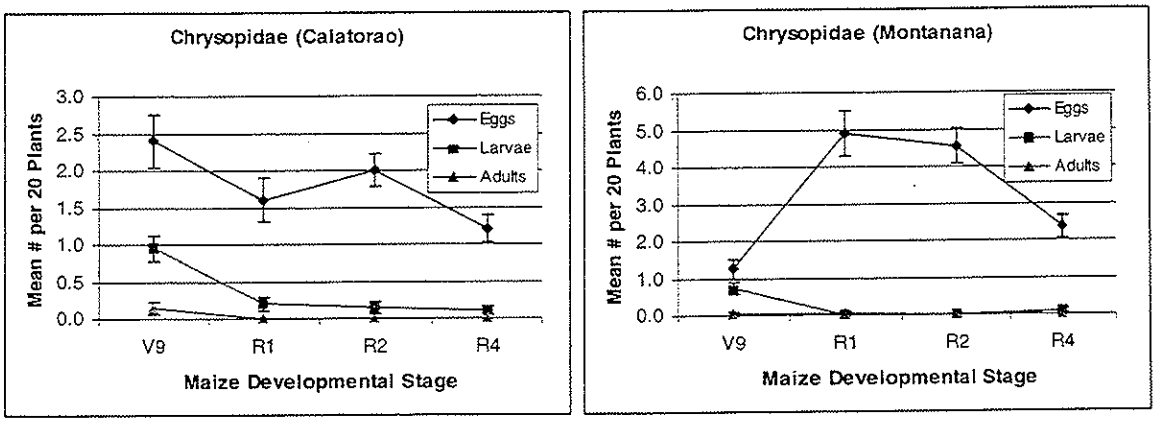


Figure 39: Mean and standard errors for Family Coccinellidae at the Spanish locations of Calatorao and Montañana

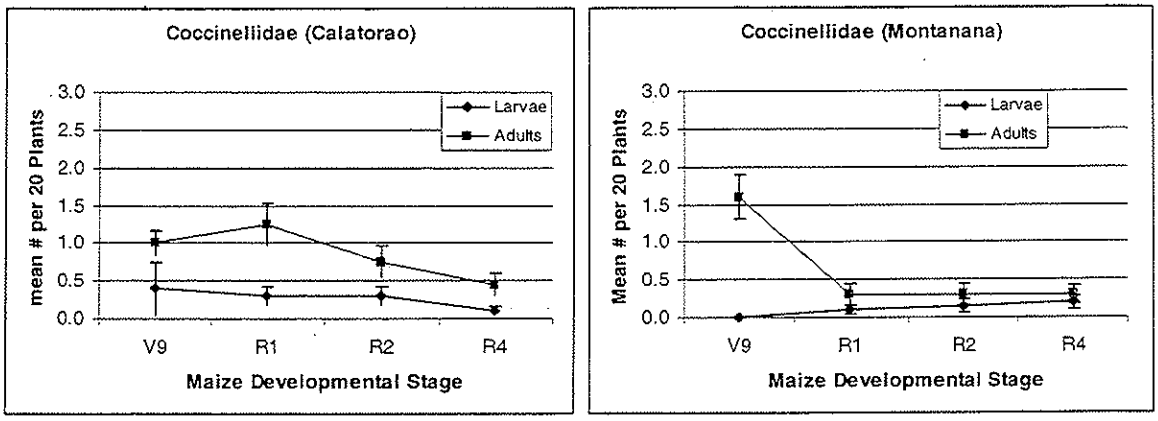
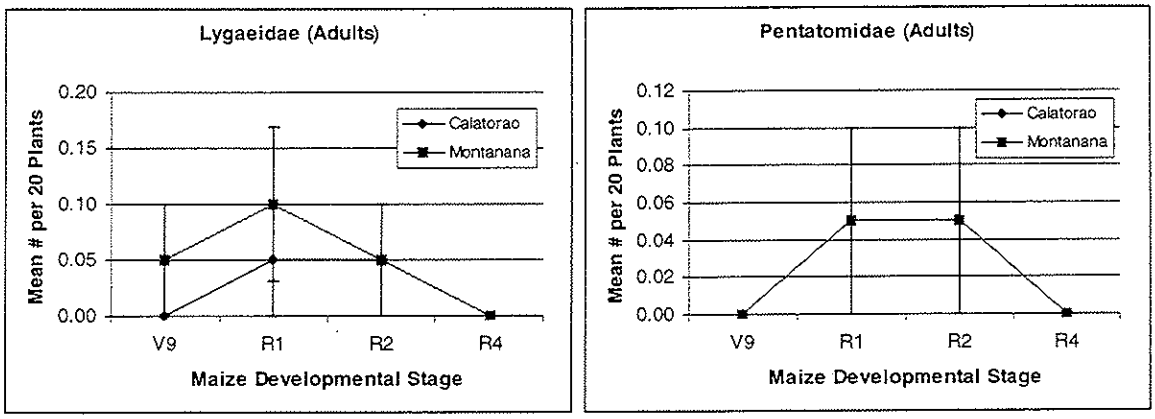


Figure 40: Means and standard errors for the families Lygaeidae and Pentatomidae at the Spanish locations of Calatorao and Montañana



TABLES

Table 1: Genetic elements in insert PHI8999A used in the transformation of 1507 maize

Location on insert PHI8999A (bp to bp incl.)	Location on plasmid PHP8999 (bp to bp incl.)	Genetic element	Size (bp incl.)	Function
1 – 80	21 – 100	Polylinker region	80	Contains restriction sites required for cloning of the genetic elements
81 – 2066	101 – 2086	*ubi*ZM1(2)	1986	The ubiquitin promoter (plus 5' untranslated region) from *Zea mays* (Christensen *et al.*, 1992)
2067 – 2089	2087 – 2109	Polylinker region	23	Contains restriction sites required for cloning of the genetic elements
2090 – 3907	2110 – 3927	*cry*1F	1818	A synthetic version of truncated *cry*1F from *Bacillus thuringiensis* sbsp. *aizawai* (plant optimized)
3908 – 3952	3928 – 3972	Polylinker region	45	Contains restriction sites required for cloning of the genetic elements
3953 – 4666	3973 – 4686	ORF25PolyA	714	A terminator from *Agrobacterium tumefaciens* pTi15955
4667 – 4722	4687 – 4742	Polylinker region	56	Contains restriction sites required for cloning of the genetic elements
4723 – 5276	4743 – 5296	CaMV 35S promoter	554	35S promoter from Cauliflower Mosaic Virus (Odell *et al.*, 1985)
5277 – 5828	5297 – 5848	*pat*	552	The synthetic glufosinate-ammonium tolerance gene (plant optimized), based on a phosphinothricin acetyltransferase gene sequence from *Streptomyces viridochromogenes* (Wohlleben *et al.*, 1988; Eckes *et al.*, 1989)
5829 – 5846	5849 – 5866	Polylinker region	18	Contains restriction sites required for cloning of the genetic elements
5847 – 6050	5867 – 6070	CaMV 35S terminator	204	35S terminator from Cauliflower Mosaic Virus (Pietrzak *et al.*, 1986)
6051 – 6235	6071 – 6255	Polylinker region	185	Contains restriction sites required for cloning of the genetic elements

Table 2: Description of the genetic elements present in the 3269 bp fragment obtained from plasmid PHP8999 that were not intended for transformation of 1507 maize

Location on plasmid PHP8999 (bp to bp incl.)	**Genetic element**	**Size (bp incl.)**	**Function**
6256 – 6307	Polylinker region	65	Contains restriction sites required for cloning of the genetic elements
6308 – 6538 (Compl.)	*lac* promoter	218	The promoter for the lac operon in E.coli; used to express cloned genes in bacteria, but non-functional in the context of PHP8999 (Blattner *et al.*, 1997). It also contains the M13 reverse primer binding site (6331 – 6354 bp, complementary)
6539 – 7493	Polylinker region	955	Contains restriction sites required for cloning of the genetic elements
7494 – 8309	*nptII*	816	Marker gene conferring kanamycin resistance (Beck *et al.*, 1982)
8310 – 8868	Polylinker region	559	Contains restriction sites required for cloning of the genetic elements
8869 – 9324 (Compl.)	Phage F1	456	The origin of replication from the bacteriophage F1; functional only in the presence of active phage F1; used to render the plasmid single-stranded DNA, rather than double-stranded DNA (Hill and Petersen, 1982).
9325 – 9504 and 1 – 20	Polylinker region	200	Contains restriction sites required for cloning of the genetic elements. It also contains the M13 forward primer binding site (9438 – 9461 bp)

Table 3: Acute sensitivity of lepidopteran species to microbially-derived CRY1F protein (Evans, 1998; Annex 25)

Insect	LC_{50} (ng CRY1F per cm^2)
European corn borer	0.58
Tobacco budworm	1.88
Fall armyworm	2.49
Corn earworm	51.6
Black cutworm	69.2

Table 4: Acute sensitivity of insect species to microbially-derived CRY1F protein (Herman and Korjagin, 1999)

Insect	LC_{50} (µg CRY1F per cm^2)
Lesser cornstalk borer	0.11
Southwestern corn borer	0.70
Sugarcane borer	1.46
Western corn borer[a]	>53.8
Corn leaf aphid[b]	>70.0
Corn leafhopper[b]	>70.0

a: No mortality at 53.8 µg CRY1F per cm^2

b: The maximum dose failed to produce more than 50% mortality on the insect species

Table 5: Comparative efficacy for 1507 maize and non-GM control maize

Maize	ECB leaf damage (1-9)[a]	ECB damage (cm tunnelling)	ECB ear damage (1-9)[a]	FAW damage (1-9)[a]	CEW damage (1-9)[a]	SWCB damage (cm tunnelling)	BCW damage (% cut-3 day)	SCB damage (% stalk damage)[b]
1507	9	1	7.8	8	6	1	32	4
Non-GM control	2	22.9	4.3	2	4	16.3	100	90

a: Damage scores are recorded visually based on a 1 to 9 scale (1 = completely susceptible, and 9 = completely resistant)
b: Least squares difference (0.05%) = 9.6

ECB = European corn borer
FAW = fall armyworm
CEW = corn earworm
SWCB = southwestern corn borer
BCW = black cutworm
SCB = sugarcane borer

Table 6: Comparative efficacy for 1507 maize (unsprayed with insecticide) and non-GM control maize (sprayed and unsprayed with insecticide) from field trials in the EU in 2000 (Vernier *et al.*, 2001b; Annex 34)

Maize[a]	ECB (number of ears damaged)	ECB + PSB (number of ears damaged)	ECB larvae per plant	PSB larvae per plant	ECB + PSB (cavities per plant)	ECB + PSB damage (cm tunnelling)	Yield (kg/ha)
1507	<1	<1	<1	0	<10	1.63	10784
Non-GM with insecticide	~5	~14	~4	~15	~45	7.29	10073
Non-GM without insecticide	~8	~12	~10	~15	~49	7.63	10171

a: Average damage by ECB and/or PSB is evaluated by examining 25 consecutive plants in each of four replicates

ECB = European corn borer
PSB = Pink stalk borer

Table 7: Summary of DNA probe sizes and locations relative to plasmid PHP8999

DNA probe	Probe size (bp)	Location on plasmid PHP8999 (bp to bp)	Comments
ubi	1587	120-1707	Hybridizes to the *ubi*ZM1(2) promoter for the *cry*1F gene
*cry*1F	979	2548-3527	Hybridizes to coding region for the *cry*1F gene
CaMV 35S	438	4790-5228	Hybridizes to the CaMV 35S promoter for the *pat* gene
pat	309	5536-5845	Hybridizes to coding region for the *pat* gene
nptII	536	7497-8033	Hybridizes to coding region for the *nptII* gene for resistance to kanamycin

Table 8: Summary of expected hybridizing fragments during Southern analyses of the DNA insert of 1507 maize

	Expected hybridizing fragment size in base pairs for each DNA probe				
Restriction enzyme	***ubi***	***cry*1F**	**CaMV 35S**	***pat***	***nptII***
*Pme*I	>6235	>6235	>6235	>6235	No fragments expected
*Hin*dIII	3890	3890	2170	2170	No fragments expected
*Pst*I	1986	914 944	1916	1916	No fragments expected
*Bam*HI	>2080	1828	1361	315 490	No fragments expected
*Eco*RI	>1467	3202	1329	1329	No fragments expected
*Bam*HI/*Eco*RI	>1467	1828	546	315 468	No fragments expected

Table 9: Summary of observed fragments during Southern analyses of the DNA insert of 1507 maize

	Observed hybridizing fragment size in base pairs for each DNA probe				
Restriction enzyme	***ubi***	***cry*1F**	**CaMV 35S**	***pat***	***nptII***
*Pme*I	~23000 [a,b]	~23000 [a,b]	~23000 [b]	~23000 [b]	No fragments observed
*Hin*dIII	~3890 [b] ~6500 [a] ~20000 [c]	~1000 [a] ~2000 [a] ~3890 [b] ~4000	~2170 [b]	~2170 [b]	No fragments observed
*Pst*I	~1986 [a,b] ~23000 [a]	~914 [b] ~944 [b] ~6500 ~23000 [a]	~1916 [b]	~1916 [b]	No fragments observed
*Bam*HI	~9000 [a] ~15000 [a] ~20000 [a]	~1828 [b] ~8000	~1361 [b]	~315 [b] ~490 [b]	No fragments observed
*Eco*RI	~1700 [a] ~3000 ~3500 ~4000 ~4100 [a] ~6500 [c] ~9400 ~23000	~3000 ~3202 [b] ~23000	~1329 [b]	~1329 [b]	No fragments observed
*Bam*HI/*Eco*RI	~1700 [a] ~3000 ~4000 [a] ~6500 [c] ~9000	~1828 [b] ~3000 ~5000 [a] ~8000	~546 [b]	~315 [b] ~468 [b]	No fragments observed

a: Similar fragment observed in negative control genomic DNA
b: DNA fragment predicted based on sequence of plasmid PHP8999
c: Detected in negative control genomic DNA only

Table 10: Summary of CRY1F protein levels (in pg/µg total extractable protein) in tissue collected from 1507 hybrid maize from field trials in Chile in 1998/99

Tissue (growth stage)[a]	Mean[b] CRY1F (pg/µg TEP[c])	Standard deviation	Min/max range (pg/µg TEP)
Leaf (V9 stage)	110.9	27.2	56.6 – 148.9
Pollen (R1 stage)	135.5	13.5	113.4 – 168.2
Silk (R1 stage)	50.3	16.5	26.8 – 79.8
Stalk (R1 stage)	550.0	104.0	355.9 – 737.4
Whole plant (R4 stage)	1063.8	361.7	803.2 – 1572.7
Grain (Physiol. maturity)	89.8	23.3	71.2 – 114.8
Senescent whole plant (Brown and dry)	714.3	95.5	622.2 – 845.3

a: Iowa State University (1997)

b: Values are means across all four sites from mean values calculated from the analysis of five individual samples per site for leaf, pollen, silk, stalk, grain and one pooled sample per site for both whole plant samples

c: TEP = total extractable protein

Table 11: Summary of CRY1F protein levels (in pg/µg total extractable protein) in tissue collected from 1507 maize from field trials in France and Italy in 1999

Tissue (growth stage)[a]	Mean[b] CRY1F (pg/µg TEP[c])	Standard deviation	Min/max range (pg/µg TEP)
Leaf (V9 stage)	348.0	160.9	193.2 – 651.4
Whole plant (V9 stage)	743.7	394.2	409.6 – 1526.6
Pollen (R1 stage)	190.5	84.4	141.9 – 630.8
Silk (R1 stage)	133.0	58.1	61.1 – 265.3
Stalk (R1 stage)	630.8	141.6	417.9 – 917.7
Whole plant (R1 stage)	671.9	348.2	323.4 – 1206.4
Whole plant (R4 stage)	1073.1[d] 569.4[e]	338.2 11.0	874.4 – 1576.1 556.7 – 575.8
Grain (Physiol. maturity)	96.4[d] 90.3[e]	25.9 21.8	44.8 – 135.3 57.4 – 131.8
Senescent whole plant (Brown and dry)	198.9	21.4	171.2 – 219.5

a: Iowa State University (1997)
b: Values are means across all sites. Samples were taken from plants not sprayed with glufosinate-ammonium unless stated otherwise
c: TEP = total extractable protein
d: Unsprayed plants
e: Sprayed with glufosinate-ammonium

Table 12: Use of inbreds to derive non-GM and GM maize hybrids with comparable genetic backgrounds

Maize hybrid	Source of hybrid		
Non-GM hybrid	Non-GM Inbred A	x	Non-GM Inbred B
GM 1507 hybrid	GM 1507 Inbred A	x	Non-GM Inbred B

Table 13: Mean agronomic data from 1507 maize and non-GM control in comparable genetic backgrounds, collected during field trials in the USA in 1999

Trait	1507 maize	Non-GM control	Number of locations	Number of replicates
Yield (kg/ha)	11510.8	11171.9	15	41
Moisture (%)	18.8	18.6	15	41
Accumulated maize growing degree days to reach 50% pollen shed	1351	1353	4	12
Accumulated maize growing degree days to reach 50% silking	1343	1337	4	12
Grain density[2]	26.47	26.42	9	27
Plant height (metres)	2.52	2.50	9	19
Ear height (metres)	1.16	1.13	9	19
Early stand count establishment (average number of plants emerging per plot)	74.5	71.7	4	12
Visual rating of emergence vigour from spike to one-leaf stage[3]	6.1	6.0	4	12
Visual rating of vigour at three- to five-leaf stage[3]	6.1	6.3	4	12
Stalk lodging[4]	0.3	0.6	11	33
Root lodging[4]	1.1	1.5	10	30
Dropped ears per plot	0.0	0.0	10	30
Top integrity[5]	7.9	7.6	9	27

1: Least Significant Difference at the 0.05 level
2: Weight (in kg) of a bushel of grain at 15.5% moisture
3: Scores are recorded visually based on a 1 to 9 scale (1 = worst, and 9 = best)
4: Average number of plants per plot that showed lodging of the specified type
5: 1-9 visual scale that describes how well the stalks remain intact above the ear, (1 = worst, and 9 =best)

Table 14: Mean agronomic data from 1507 maize and non-GM control in comparable genetic backgrounds, collected during field trials in France (3 locations), Italy (2 locations) and Bulgaria (1 location) in 2000. Total number of replicates was n = 18

Trait	1507 maize	1507 maize sprayed[1]	Non-GM control
Moisture (%)	33	33	33
Accumulated heat units to reach 50% pollen shed	915	917	911
Accumulated heat units to reach 50% silking	893	896	894
Plant height (metres)	2.48	2.41	2.40
Ear height (metres)	1.08	1.04	1.05
Final population (total number of viable plants remaining at maturity)	131	127	125
Stay green[2]	5	5	5
Stalk lodging[3]	0	0	2
Root lodging[3]	0	0	1
Disease incidence[4]	8	8	8
Insect damage[5]	9	9	8

1: Sprayed with glufosinate-ammonium herbicide
2: Overall plant health at maturity on a 1 to 9 scale where 1 is completely dead and 9 is very green
3: Average number of plants per plot that showed lodging of the specified type
4: Level of disease resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no visible disease
5: level of destructive insect resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no damage

Table 15: Mendelian segregation of 1507 maize. Early segregation data obtained from the BC2F1 generation; later segregation data obtained from the F1 generation (see Figure 19)

Generation	Observed ratio[a]	Expected ratio	Chi Square	Alpha value	Significant difference?[b]
BC2F1	248 : 278	263 : 263	1.711	0.1909	No
F1	910 : 493	935.3 : 467.7	2.903	0.0884	No

a: Data expressed as number of observed tolerant to glufosinate-ammonium : number of observed plants susceptible to glufosinate-ammonium herbicide
b: Significant at alpha = 0.05

Table 16: Comparison of the biological activity of maize expressed CRY1F protein (event 1360) and microbially-derived CRY1F protein (Evans, 1998; Annex 25)

Insect	LC_{50} (ng CRY1F per cm^2)	
	Maize expressed CRY1F	**Microbially-derived CRY1F**
European corn borer	0.58	0.58
Tobacco budworm	1.74	1.88
Fall armyworm	2.21	2.49
Corn earworm	>15.8[a]	51.6
Black cutworm	22.7[a]	69.2

a: The maximum dose failed to produce more than 50% mortality on the insect species. The LC_{50}s were extrapolated beyond the actual data

Table 17: Nutritional equivalence of 1507 maize: poultry feeding study (Annex 5)

Parameter	Treatment					
	Control (Bin #1)	Control (Bin #2)	Control (Bin #3)	Control (Bin #4)	Non-GM control hybrid (7250)	1507 maize
Mortality (%)	5.71	5.71	2.86	5.71	2.86	5.71
Stats.[a]	a	a	a	a	a	a
Body Weight (kg) Day 0	0.044	0.043	0.043	0.043	0.044	0.043
Stats.[a]	a	a	a	a	a	a
Body Weight (kg) Day 42	1.730	1.739	1.738	1.728	1.739	1.757
Stats.[a]	a	a	a	a	a	a
Daily Gain (g per bird per day)	0.040	0.040	0.040	0.040	0.040	0.041
Stats.[a]	a	a	a	a	a	a
Feed Conversion (Body weight corrected)	1.797	1.806	1.808	1.804	1.802	1.775
Stats.[a]	a	a	a	a	a	a

a: Treatment means within a row without a common letter are significantly different ($p < 0.05$)

Table 18: Summary of sequence for 1507 maize insert. The ORF3 sequence extends from base 1896 to base 2648 of the 5' border sequence, spanning regions 4 to 7b

Region	Location in 1507 insert	Size (bp)	% Identity	Homologue	Location in homologous sequence	Description
1	1-669	669	N/A[1]	N/A	N/A	No known homology
2	670-869	200	90.5	AF123535	52432-52632 (complement)	Undescribed maize genomic sequence
3	870-1681	812	89.4	AF050439	1-801	Fragment of maize Huck-1 retrotransposon 5' LTR[2]
			86.6	AF050438	1-797	Fragment of maize Huck-1 retrotransposon 3' LTR
4	1682-2016	335	100.0	PHI8999A	3149-3483	Fragment of *cry*1F gene
5	2017-2337	321	100.0	X86563	29429-29749	Fragment of maize chloroplast *rpoC2* gene (RNA polymerase beta-2 subunit)
6	2338-2354	17	100.0	X86563	97643-97659	Fragment of maize chloroplast *trnI* gene (tRNA-Ile)
			82.4	PHI8999A	182-197	Fragment of maize *ubi*ZM1(2) promoter
7a	2358-2558	201	100.0	PHI8999A	5320-5475	Fragment of *pat* gene
7b	2559-2696	138	99	PHI8999A	5336-5518 (complement)	Fragment of *pat* gene
7c	2697-2711	15	100.0	PHI8999A	2544-2558 (complement)	Fragment of *cry*1F gene
8	2712-2829	118	100.0	PHI8999A	36-153	Fragment of polylinker region (bases 36-80) and *ubi*ZM1(2) promoter (bases 81-153)
9	2830-9015	6186	100.0	PHI8999A	11 – 6196	Full-length insert of PHI8999A
10	9016-9565	550	100.0	PHI8999A	3906-4456 (complement)	Inverted ORF25PolyA terminator and upstream polylinker sequence
11	9566-9693	128	100.0	NC_001666	121851-121978 (complement) & 100759-100886	Fragment of maize chloroplast *rps12* rRNA (23S ribosomal RNA)
12	9696-10087	392	99	NC_001666	17091-17483 (complement)	Fragment of maize chloroplast genome
13	10088-10275	188	99	PHI8999A	5333-5520 (complement)	Fragment of *pat* gene
14	10278-10358	81	100	NC_001666	137122-137202 (complement)	Fragment of maize chloroplast "ORF241" – hypothetical protein gene
15	10359-10629	271	N/A[1]	N/A	N/A	No known homology

[1]_ N/A; not applicable
[2]_ LTR; long terminal repeat

Table 19: PCR primers for regions 1 to 3 at the 1507 maize insert 5' border sequence and for two regions unique to the 1507 maize insert

Reaction	PCR amplicon location	Amplicon size (bp)	Region in 1507 maize 5' border sequence	Amplicon present in control maize	Amplicon present in 1507 maize
A	In 1507 5' border sequence	300	Region 1	Yes	Yes
B	In 1507 5' border sequence	768	Region 1 to region3	Yes	Yes
C	In 1507 5' border sequence	424	Region 2 to region 3	Yes	Yes
D	PHI8999A sequence in insert	194	Spans CaMV35S term to inverted ORF25 terminator on 3' end (region 10)	No	Yes
E	*cry*1F fragment in 5' border sequence	366	Spans 335 bp *cry*1F fragment in 5' border sequence (region 4)	No	Yes

Table 20: PCR primers for regions 13 to 15 at 1507 maize insert 3' border sequence

Reaction	PCR amplicon location	Amplicon size (bp)	Region in 1507 maize 3' border sequence	Amplicon present in control maize	Amplicon present in 1507 maize
F	In 1507 3' border sequence	342	Region 13 (*pat* gene fragment) to region 15	No	Yes
G	In 1507 3' border sequence	252	Region 14 (chloroplast gene) to region 15	No	Yes
H	In 1507 3' border sequence	175	Region 15	Yes	Yes
I	In 1507 3' border sequence	134	Region 15	Yes	Yes
J	In 1507 3' border sequence	107	Region 15	Yes	Yes

Table 21: Summary of DNA probe sizes and locations relative to plasmid PHP8999

Probe name	Probe size (bp)	Location on plasmid PHP8999(bp to bp)	Comments
*cry*1F	980	2548 – 3527	Hybridizes to coding region for the *cry*1F gene
Full-length *pat*	548	5284 – 5831	Hybridizes to the complete coding region for the *pat* gene
3' *pat*	310	5536 – 5845	Hybridizes to the 3' end of the coding region for the *pat* gene
Backbone Probe 25	1245	6256 – 7500	Hybridizes to backbone sequence of PHP8999 outside of the *Pme*I fragment used for transformation
kan	625	7494 – 8118	Hybridizes to coding region for the *nptII* gene for resistance to kanamycin
Backbone Probe 34	1476	8010 - 9485	Hybridizes to backbone sequence of PHP8999 outside of the *Pme*I fragment used for transformation

Table 22: Groups of rats involved in the 90-day oral toxicity study together with the diets fed to each of those groups

Group		Number/Group		
Male	**Female**	**Male**	**Female**	**Diet Concentrations[a]**
I	II	12	12	33% transgenic maize (33% 1507)
III	IV	12	12	33% near isogenic maize (33% 33P66)
V	VI	12	12	33% commercial maize (33% 33J56)
VII	VIII	12	12	11% transgenic maize (11% 1507)[b]
IX	X	12	12	11% near isogenic maize (11% 33P66)[b]

a Weight of test maize/Total diet weight.
b These diets also contain 22% 33J56.

Table 23: Comparative efficacy for 1507 maize and non-GM control maize from field trials in Spain in 2002

		Montañana[1]		Calatorao[2]	
Entry No.	**Event**	**Rep. 1**	**Rep. 2**	**Rep. 1**	**Rep. 2**
1	1507 maize A	1.5	0.5	4.3	4.9
2	Non-GM maize A	16.2	13.6	64.3	45.2
3	1507 maize B	0.6	1.1	7.8	9.3
4	Non-GM maize B	23.7	18.0	65.5	64.8
5	1507 maize C	5.2	13.7	17.4	35.8
6	Non-GM maize C	16.1	22.1	66.9	88.0
7	1507 maize D	4.8	0.9	5.2	7.2
8	Non-GM maize D	26.2	16.3	63.1	63.0
9	Non-GM maize E	21.8	14.9	64.5	73.2
10	Non-GM maize F	19.1	28.1	61.4	62.5
Mean 1507		3.5		11.5	
Mean non-GM		19.0		65.1	

[1] Mean insect tunnelling length (cm) in the stalks of samples of 10 plants per plot; at Montañana 50% of the larvae were identified as ECB and 50% as *Sesamia* spp.

[2] Mean insect tunnelling length (cm) in the stalks of samples of 10 plants per plot; at Calatorao 5% of the larvae were identified as ECB and 95% as *Sesamia* spp.

Table 24: Agronomic data obtained from field trials of 1507 maize in Spain in 2002. Summary of germination, time to pollen shed, time to silking, stalk lodging, root lodging, plant height, and ear height. The data was obtained from three locations and total number of replicates was n = 9

	Germination/ early population[1]	GDU[8] 50% pollen shed[2]	GDU[8] 50% silking[3]	Stalk lodging[4] (%)	Root lodging[5] (%)	Plant height[6] (cm)	Ear height[7] (cm)
Montañana (Aragón, Spain)							
1507 maize	41	814.5	888.7	1	0	191	63
Non-GM control	38	788.7	858.5	17	0	180	57
Cogullada (Aragón, Spain)							
1507 maize	45	815.5	890.5	2	0	193	64
Non-GM control	49	774.6	845	32	0	186	60
Calatorao (Aragón, Spain)							
1507 maize	56	840.9	915.9	2	0	208	85
Non-GM control	58	788.7	855.8	49	0	193	76
Average							
1507 maize	47	823.6*	898.4*	2*	0	197*	71*
Non-GM control	48	784.0	853.1	33	0	186	64

[1] Number of plants emerged per 60 seed planted
[2] Number of accumulated heat units when approximately 50% of the plants are shedding pollen
[3] Number of accumulated heat units when approximately 50% of the plants are silking
[4] Percent of plants broken below the primary ear
[5] Percent of plants leaning ≥ 30° in the first ½ meter above the soil surface
[6] Measured from the soil surface to the tip of tassel), n=10
[7] Measured from the soil surface to the base primary ear), n=10
[8] GDU: Growing Degree Units or accumulated heat units
* Statistically significant differences (P-value < 0.05)

Table 25: Agronomic data obtained from field trials of 1507 maize in Spain in 2002. Summary of final population, stay green, disease incidence, insect damage, and grain moisture. The data was obtained from three locations and total number of replicates was n = 9

	Final population[1]	Stay green[2]	Disease incidence[3]	Insect damage[4]	Grain moisture[5]
Montañana (Aragón, Spain)					
1507 maize	14	3	8	9	29
Non-GM control	15	2	9	6	29
Cogullada (Aragón, Spain)					
1507 maize	19	1	8	8	26
Non-GM control	11	1	9	3	26
Calatorao (Aragón, Spain)					
1507 maize	15	2	9	8	34
Non-GM control	11	1	9	4	32
Average					
1507 maize	16	2	8	8*	30
Non-GM control	12	1	9	4	29

[1] Total number of viable plants (per plot) remaining at maturity
[2] Overall plant health at maturity evaluated on a 1 to 9 scale where 1 is completely dead and 9 is very green
[3] Level of disease resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no visible disease
[4] Level of destructive insect resistance at maturity evaluated on a 1 to 9 scale where 1 is poor resistance and 9 is high resistance or no damage
[5] Percent water content of grain at typical harvest maturity
* Statistically significant differences (P-value < 0.05)

Table 26: Comparative agronomic data from different hybrids of 1507 maize and non-GM control maize from field trials in Spain in 2002. The data was obtained from two locations and total number of replicates was n = 4

Entry No.	Event	Yield (kg/ha)	Yield (% of mean)[1]	Moisture at harvest (%)	Root lodging[2]	Stalk lodging[3]	ECB + *Sesamia* damage[4]
1	1507 maize A	9696.8	120.2	28.4	0	2	8.7
2	Non-GM maize A	7380.9	92.0	27.5	0	21	4.7
3	1507 maize B	9445.8	117.3	27.8	8	2	8.0
4	Non-GM maize B	7851.6	97.6	27.6	5	19	5.7
5	1507 maize C	8987.6	112.0	26.7	1	1	7.5
6	Non-GM maize C	8134.0	102.0	28.1	0	20	4.7
7	1507 maize D	8203.1	102.3	28.1	4	9	6.7
8	Non-GM maize D	7437.4	93.0	25.6	2	15	5.5
9	Non-GM maize E	6470.8	80.8	28.3	2	22	5.0
10	Non-GM maize F	6621.4	82.8	26.0	13	21	4.0

[1] Yield expressed as percent of overall experiment mean; overall mean is 8022.9 kg/ha
[2] Count of early (before flowering) root lodged plants in two central rows
[3] Number of stalk lodged plants in two central rows; considered plants broken at or below ear node
[4] Damage scores are recorded visually based on a 1 to 9 scale (1 = completely susceptible, and 9 = completely resistant)

Table 27: Means and standard errors of non-target species found on control and 1507 maize hybrids for Calatorao and Montañana in 2002. Control estimates result from pooling 20 observations per plot, 2 plots per hybrid entry and 6 entries per location (240 individual plant observations per location), and 1507 estimates result from pooling 20 observations per plot, 2 plots per hybrid entry and 4 entries per location (160 individual plant observations per location)

Maize Stage	Maize Type*		Coccinellidae		Nabidae		Anthocoridae		Chrysopidae			Pentatomidae		Lygaeidae	
		Spider	Larvae	Adults	Nymphs	Adults	Nymphs	Adults	Eggs	Larvae	Adults	Nymphs	Adults	Nymphs	Adults
		Calatorao, Spain													
V9	control	3.6 ± 0.47	0.7 ± 0.58	0.8 ± 0.21	1.1 ± 0.29	1.3 ± 0.26	5.3 ± 1.06	49.9 ± 3.04	2.5 ± 0.48	0.8 ± 0.13	0.1 ± 0.08	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
V9	1507	2.9 ± 0.60	0.1 ± 0.03	1.1 ± .020	1.1 ± 0.17	1.3 ± 0.28	4.8 ± 1.05	42.7 ± 6.63	1.9 ± 0.43	1.0 ± 0.29	0.2 ± 0.12	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
R1	control	4.5 ± 0.56	0.4 ± 0.19	1.3 ± 0.33	2.3 ± 0.22	2.3 ± 0.37	19.2 ± 2.01	55.9 ± 2.43	1.6 ± 0.43	0.3 ± 0.14	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.08	0.0 ± 0.00	0.0 ± 0.00
R1	1507	4.2 ± 0.55	0.1 ± 0.09	1.2 ± 0.37	1.9 ± 0.34	1.5 ± 0.31	14.4 ± 2.62	46.9 ± 5.75	1.5 ± 0.28	0.2 ± 0.09	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.09
R2	control	4.3 ± 0.64	0.3 ± 0.19	0.7 ± 0.22	2.8 ± 0.37	1.7 ± 0.36	15.4 ± 1.18	51.3 ± 2.98	2.0 ± 0.30	0.2 ± 0.11	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.08	0.0 ± 0.00	0.0 ± 0.00
R2	1507	4.0 ± 0.48	0.2 ± 0.11	0.9 ± 0.31	1.7 ± 0.33	1.7 ± 0.24	16.4 ± 2.10	41.4 ± 4.62	1.7 ± 0.31	0.1 ± 0.09	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.09
R4	control	1.6 ± 0.31	0.1 ± 0.08	0.2 ± 0.11	2.5 ± 0.31	3.3 ± 0.46	9.7 ± 1.04	27.8 ± 1.53	1.2 ± 0.21	0.1 ± 0.08	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
R4	1507	1.6 ± 0.33	0.2 ± 0.09	0.8 ± 0.21	1.9 ± 0.40	2.6 ± 0.53	8.0 ± 1.12	24.1 ± 2.90	1.2 ± 0.27	0.1 ± 0.09	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
		Montañana, Spain													
V9	control	3.9 ± 0.54	0.0 ± 0.00	2.1 ± 0.38	2.0 ± 0.58	1.3 ± 0.28	13.4 ± 1.36	45.8 ± 4.47	1.6 ± 0.31	0.8 ± 0.22	0.1 ± 0.08	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
V9	1507	3.1 ± 0.81	0.1 ± 0.04	0.7 ± 0.22	1.6 ± 0.29	0.9 ± 0.25	11.7 ± 1.44	38.6 ± 6.18	0.9 ± 0.29	0.6 ± 0.20	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.09
R1	control	4.6 ± 0.60	0.2 ± 0.11	0.4 ± 0.23	2.8 ± 0.72	2.2 ± 0.34	31.8 ± 2.11	78.6 ± 5.82	5.1 ± 0.88	0.0 ± 0.00	0.1 ± 0.08	0.0 ± 0.00	0.1 ± 0.08	0.0 ± 0.00	0.2 ± 0.11
R1	1507	3.7 ± 0.55	0.0 ± 0.00	0.3 ± 0.12	1.3 ± 0.32	2.0 ± 0.36	23.0 ± 4.25	60.9 ± 7.79	3.7 ± 0.74	0.2 ± 0.11	0.1 ± 0.04	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
R2	control	4.0 ± 0.37	0.1 ± 0.08	0.3 ± 0.18	2.5 ± 0.42	3.2 ± 0.63	24.0 ± 2.69	53.3 ± 3.49	4.5 ± 0.63	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.08	0.0 ± 0.00	0.0 ± 0.00
R2	1507	2.6 ± 0.34	0.2 ± 0.12	0.3 ± 0.18	2.3 ± 0.46	3.1 ± 0.46	22.1 ± 3.28	50.2 ± 6.20	4.0 ± 0.62	0.1 ± 0.04	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.09
R4	control	2.2 ± 0.34	0.2 ± 0.11	0.4 ± 0.19	2.3 ± 0.39	2.3 ± 0.48	9.7 ± 0.97	36.2 ± 2.86	2.4 ± 0.43	0.2 ± 0.11	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
R4	1507	2.0 ± 0.31	0.2 ± 0.11	0.2 ± 0.10	2.5 ± 0.40	2.2 ± 0.32	14.0 ± 1.52	29.6 ± 3.31	2.3 ± 0.38	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00

* control = non-transgenic hybrid entries (2 official check hybrids and 4 hybrids that are isogenic to the TC1507 hybrids); 1507 = transgenic hybrid entries (4 hybrids expressing the Cry1F protein).

Table 28: Tryptic peptide mass data (*m/z* $[M+H]^+$) of microbially-derived and 1507 maize expressed CRY1F proteins obtained by MALDI-TOF MS (Schafer and Schwendler, 2001; Annex 17)

Full Length CRY1F residue No.	**Theoretical mass (*m/z*)**	**Microbially-derived CRY1F [M+H]**	**1507 maize expressed CRY1F [M+H]**
32-42	1227.72	1227.70	1227.68
100-113	1612.81	1612.81	1612.79
114-125	1441.67	1441.66	1441.65
172-193	2434.15	2434.21	2434.16
194-200	878.55	878.51	878.50
204-217	1675.79	1675.75	1675.75
252-263	1394.72	1394.69	1394.68
264-286	2509.21	2509.24	2509.19
312-324	1413.71	1413.70	1413.68
358-366	1033.56	1033.52	1033.52
367-379	1386.71	1386.70	1386.69
380-392	1416.68	1416.67	1416.67
431-442	1376.62	1376.62	1376.59
452-463	1301.63	1301.60	1301.58
464-471	911.58	911.53	911.52
472-483	1269.68	1269.66	1269.65
484-494	1089.56	1089.53	1089.52
522-529	925.46	ND[b]	925.43
530-538	1007.54	1007.51	1007.50
539-546	924.48	924.44	924.43

Note:

[a] Two digit decimals were used for mass data in this table although raw data obtained from the MALDI-TOF-MS spectrometer were shown in 4 digit decimals. A peptide was considered a match if its *m/z* is within *m/z* 0.1 error range of its theoretical *m/z*.

[b] ND: not detected.

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SECTION 3

ADDITIONAL INFORMATION (ANNEX IV, DIRECTIVE 2001/18/EC)

A. The following information shall be provided in the notification for placing on the market of GMOs as or in products in addition to that of Annex III (Directive 2001/18/EC):

1. Proposed commercial names of the products and names of GMOs contained therein, and any specific identification, name or code used by the notifier to identify the GMO

As mentioned in Point **A.1.c.** of **Section 2** commercial names will be assigned to 1507 maize seed at the time of market introduction. The product described in this notification is *B.t.* Cry1F maize line 1507, referred to as 1507 maize. It consists of maize product consisting of or derived from seed of 1507 maize genetically modified to express CRY1F protein, conferring resistance to certain lepidopteran insect pests, and PAT protein, conferring tolerance to glufosinate-ammonium herbicide. The maize product also consists of progeny derived from conventional breeding between 1507 maize with any traditionally bred maize.

In accordance with the OECD guidance for the designation of a unique identifier for transgenic plants (ENV/JM/MONO(2002)7), the unique identification code assigned to 1507 maize is DAS-Ø15Ø7-1.

2. Name and full address of the person established in the Community who is responsible for the placing on the market, whether it be the manufacturer, the importer or the distributor

This is a joint notification submitted by Pioneer Hi-Bred International Inc., as represented by Pioneer Overseas Corporation, and Mycogen Seeds, c/o Dow AgroSciences LLC. Pioneer Hi-Bred is the primary contact for this submission and therefore all correspondence should be sent to the responsible scientist at Pioneer Overseas Corporation:

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3. Name and full address of the supplier(s) of control samples

A PCR detection method to confirm the molecular identity of 1507 maize has been developed by GeneScan Analytics GmbH and is attached as Annex 15. Provided that Pioneer Hi-Bred International Inc. and Mycogen Seeds, c/o Dow AgroSciences LLC intellectual property rights are protected, a reasonable amount of 1507 maize control samples as reference material will be made available to the regulatory authority before placing 1507 maize products on the EU market.

We propose to work with a central body such as the EC Institute for Reference Materials and Measurements as the supplier of control samples. Further information will be provided in due course.

4. Description of how the product and the GMO as or in product are intended to be used. Differences in use or management of the GMO compared to similar non-genetically modified products should be highlighted

Products from 1507 maize will be used in a manner consistent with current uses of maize grain and maize products and in accordance with the monitoring plan (see **Section 5** of this notification) and insect resistance management (IRM; Annex 37) strategy developed in the context of responsible product stewardship for the cultivation of 1507 maize. Use of 1507 maize will include cultivation and import of grain and grain products for storage and processing into food, animal feed and industrial uses. The approval for food use of 1507 maize is being considered separately in accordance with Regulation (EC) No 258/97.

Maize, together with rice and wheat, is one of the most important cereal crops in the world with total production of 596.4 million tonnes in 2000 (FAOSTAT Database, 2000). The FAO estimation for the EU maize production in 2000 is 38.4 million tonnes. Majority of grain and forage derived from maize is used for animal feeds, and about 8% of the grain is processed for human food products mainly by wet-milling or dry-milling. Maize grain is also processed into industrial products (11%), such as ethyl alcohol by fermentation and highly refined starch by wet-milling to produce starch and sweetener products. In addition to milling, the maize germ can be processed to obtain maize oil.

5. **Description of the geographical area(s) and types of environment where the product is intended to be used within the Community, including, where possible, estimated scale of use in each area**

Maize is widely cultivated in a variety of agricultural environments of the EU, with strong variations at a regional level throughout the Community. In 2000, annual production of maize grain in the EU was estimated to be 38.4 million tonnes (FAOSTAT Database, 2000). The largest producer is France (43%), followed by Italy (25%), Spain (11%) and Germany (8%). The majority (80%) of grain and forage derived from maize is used for animal feed. However, about 3.3 million tonnes of the grain (or 8% of EU production) is processed for human food products. Grain and derived products from 1507 maize are expected to be part of this production.

6. **Intended categories of users of the product e.g. industry, agriculture and skilled trades, consumer use by public at large**

As mentioned in Point **A.4.** above, 1507 maize will be used in the EU as any other maize. Therefore, there are multiple categories of users of 1507 maize, including the animal feed and milling industry, agriculture, skilled trades and consumer use by public at large.

7. **Information on the genetic modification for the purposes of placing on one or several registers of modifications in organisms, which can be used for the detection and identification of particular GMO products to facilitate post-marketing control and inspection**

The 1507 maize has been genetically modified (GM) to express the proteins CRY1F and phosphinothricin-N-acetyltransferase (PAT). Expression of the CRY1F protein confers season-long resistance against certain lepidopteran pests, such as the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. It is expressed constitutively and when cultivated, it provides control against insect pest damage.

Expression of the PAT protein confers tolerance to application of glufosinate-ammonium herbicide. It is expressed constitutively serving as a selectable marker and when cultivated, the maize plant will tolerate field application rates of 1600 g a.i./ha of glufosinate-ammonium without showing any phytotoxicity symptoms. Tolerance to the herbicide provides for an alternative in weed management.

A PCR detection method unique for the 1507 maize event has been developed for the purposes of detection and identification to facilitate post-marketing monitoring control and inspection (Annex 15). Provided that Pioneer Hi-Bred International, Inc. and Mycogen Seeds c/o Dow AgroSciences LCC intellectual property rights are protected, a reasonable amount of 1507 maize reference material will be made available to the regulatory authority before placing 1507 maize products on the EU market. It is preferable that the PCR detection method and reference material is given to a central body operating under the auspices of the EU regulatory authorities and the European Commission.

8. Proposed labelling on a label or in an accompanying document

Product information to indicate that genetic modification has been used in the development of 1507 maize will be provided on a label and/or in an accompanying document in accordance with Annex IV of Directive 2001/18/EC (Point **B.7.** below). This will enable products from 1507 maize to be labelled in accordance with Directive 2001/18/EC.

A proposal for the labeling of products consisting of, or containing, genetically modified 1507 maize is attached to this notification as **Section 6**.

B. The following information shall be provided in the notification, when relevant, in addition to that of Point A:

1. Measures to take in case of unintended release or misuse

This notification is for consent to market genetically modified 1507 maize products in accordance with Part C of Directive 2001/18/EC. The scope of this notification is for all uses of 1507 maize including cultivation of 1507 maize seed (inbreds and hybrids) in the EU. The proposed uses of grain and other products of 1507 maize, arising from imports or cultivation, will be the same as for any other maize. The use of 1507 maize for human food is considered in a separate application submitted in accordance with Regulation (EC) No. 258/97.

The conclusions from the environmental risk assessment (e.r.a.) for the placing on the market of 1507 maize in accordance to Annex II of Directive 2001/18/EC (**Section 4** of this notification) confirm that there is no risk to human and animal health or the environment arising from 1507 maize. In addition, there is no significant risk to non-target organisms. However, the e.r.a. does identify a limited potential risk posed by the cultivation of 1507 maize due to the potential development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population, and therefore an insect resistance management (IRM; Annex 37) strategy is proposed for the cultivation of 1507 maize in the context of product stewardship.

Any unintentional release or misuse of 1507 maize can be controlled by current agronomic practices including cultivation, selective use of herbicides (with the exception of glufosinate-ammonium) and crop rotation.

2. Specific instructions or recommendations for storage and handling

The safety evaluation in this notification confirms that no specific instructions for storage and handling are necessary for the placing on the market of 1507 maize. Therefore, products of 1507 maize will be stored and handled in the same way as products from other commercial maize varieties.

3. Specific instructions for carrying out monitoring and reporting to the notifier and, if required, the competent authority

Specific instructions for carrying out monitoring and reporting to the notifier and, if required, the competent authority have been described in detail in the proposal for a monitoring plan attached to this notification as **Section 5** and in accordance with Annex VII of Directive 2001/18/EC.

4. Proposed restrictions in the approved use of the GMO

The safety evaluation in this notification confirms that no specific restrictions are necessary for the placing on the market of 1507 maize, and seeds for cultivation and products of 1507 maize will be used in the same way as seeds

and products from other commercial maize varieties. Specific conditions of use will apply in case of cultivation of 1507 maize seeds as described in detail in the proposed monitoring plan (see **Section 5** of this notification).

The period of the first consent to place on the market 1507 maize is requested for the maximum period and should end ten years after the date of the first inclusion of the first plant variety containing the GMO on an official national catalogue of plant varieties in accordance with Council Directives 70/457/EEC and 70/458/EEC.

5. Proposed packaging

The packaging, storage, and handling systems that are currently used for maize will apply. The grain and processed products of 1507 maize will be packaged in the same manner as other maize products.

6. Estimated production in and/or imports to the Community

In 2000, annual production of maize grain in the EU has been estimated at 38.4 million tonnes (FAOSTAT Database, 2000). The largest producer is France (43%), followed by Italy (25%), Spain (11%) and Germany (8%). Majority (80%) of grain and forage derived from maize is used for animal feed. However, about 3.3 million tonnes of the grain (or 8% of EU production) is processed for human food products with highest consumption in the UK (34%), followed by Italy (21%), Germany (20%) and France (11%) (EUROSTAT New Cronos, 2000). Import of maize grain into the EU for 1999 has been estimated to be of about 2 million tonnes (EUROSTAT New Cronos, 2000). Production and/or import of 1507 maize into the Community is expected to be part of the above production and import estimates.

7. Proposed additional labelling

As mentioned in Point **A.8.** above, product information to indicate that genetic modification has been used in the development of 1507 maize will be provided on a label and/or in an accompanying document in accordance with Annex IV of Directive 2001/18/EC (see **Section 6** of this notification). This will enable products from 1507 maize to be labelled in accordance with Directive 2001/18/EC. Additional information on 1507 maize will also be provided in an accompanying document to agricultural users to indicate specific conditions of use of 1507 maize in the EU.

In accordance with the OECD guidance for the designation of a unique identifier for transgenic plants (ENV/JM/MONO(2002)7), the unique identification code assigned to 1507 maize is DAS-Ø15Ø7-1.

In accordance with Annex IV of Directive 2001/18/EC, the proposed additional labelling of 1507 maize will include, at least in summarized form, the information from **Points A.4.**, **A.5.**, **B.1.**, **B.2.**, **B.3.** and **B.4.** of this **Section 3**.

SECTION 4

ENVIRONMENTAL RISK ASSESSMENT FOR THE PLACING ON THE MARKET OF 1507 MAIZE IN ACCORDANCE WITH ANNEX II OF DIRECTIVE 2001/18/EC

Introduction

The product described in this notification is *B.t.* Cry1F maize line 1507, referred to as 1507 maize. It consists of maize product consisting of or derived from seed of 1507 maize genetically modified to express CRY1F protein, conferring resistance to certain lepidopteran insect pests, and PAT protein, conferring tolerance to glufosinate-ammonium herbicide. The maize product also consists of progeny derived from conventional breeding between 1507 maize with any traditionally bred maize.

This notification is for consent to market genetically modified 1507 maize products in accordance with Part C of Directive 2001/18/EC. The scope of this notification is for all uses of 1507 maize including cultivation of 1507 maize seed (inbreds and hybrids) in the EU. The proposed uses of grain and other products of 1507 maize, from imports or cultivation, will be the same as for any other maize.

An environmental risk assessment (e.r.a.) for the placing on the market of 1507 maize has been carried out in accordance with Commission Decision 2002/623/EC establishing the guidance notes supplementing Annex II of Directive 2001/18/EC. The e.r.a. takes into account the relevant information provided throughout this notification.

Food use of 1507 maize is considered in a separate application submitted on 15 February 2001 to the Competent Authority of The Netherlands in accordance with Regulation (EC) No. 258/97.

A. OBJECTIVE

The objective of an environmental risk assessment (e.r.a.) is, on a case by case basis, to identify and evaluate potential adverse effects of the genetically modified organism (GMO), either direct and indirect, immediate or delayed, on human health and the environment which the deliberate release or the placing on the market of GMOs may have. The e.r.a. for the placing on the market of 1507 maize has been conducted with a view to identifying if there is a need for risk management and if so, the most appropriate methods to be used.

B. GENERAL PRINCIPLES

In accordance with the precautionary principle, the following general principles have been followed when performing the e.r.a. for the placing on the market of 1507 maize:

- Identified characteristics of the GMO and its use which have the potential to cause adverse effects should be compared to those presented by the non-modified organism from which it is derived and its use under corresponding situations;

- The e.r.a. should be carried out in a scientifically sound and transparent manner based on available scientific and technical data;

- The e.r.a. should be carried out on a case by case basis, meaning that the required information may vary depending on the type of the GMOs concerned, their intended use and the potential receiving environment, taking into account, i.a., GMOs already in the environment;

- If new information on the GMO and its effects on human health or the environment becomes available, the e.r.a. may need to be readdressed in order to:

  - o Determine whether the risk has changed;

  - o Determine whether there is a need for amending the risk management accordingly.

C. METHODOLOGY

C.1. CHARACTERISTICS OF THE GMO AND RELEASES

The e.r.a. for the placing on the market including cultivation of *B.t.* Cry1F maize line 1507, referred to as 1507 maize, has being carried out taking into account the relevant technical and scientific details regarding the specific characteristics of 1507 maize and its proposed uses as described below.

a) The recipient or parental organism

The recipient organism for the genetic modification of 1507 maize is the Gramineae *Zea mays* L., commonly known as maize in the EU or corn in the USA. The genetic modification was carried out on the non-GM maize line Hi-II, as described in detail in Point **C.1.** of **Section 2** of this notification and summarized in Point **C.1.b.**, below.

Detailed information on the recipient organism has been provided in **Part B** of **Section 2** of this notification. It includes information concerning maize reproduction (Point **B.2.** of **Section 2**), survivability (Point **B.3.** of **Section 2**), dissemination (Point **B.4.** of **Section 2**), geographical distribution (Point **B.5.** of **Section 2**), habitat (Point **B.6.** of **Section 2**), and interactions with other organisms, including potential effects on human health (Point **B.7.** of **Section 2**).

In line with the general principles (Point **B.**, above), the relevant information on the characteristics of the non-GM recipient organism and its use under corresponding

situations serves as the baseline for comparison to 1507 maize during consideration of each consecutive step of the e.r.a. for placing on the market of 1507 maize.

b) The genetic modification and relevant information on the vector and the donors

The 1507 maize has been genetically modified to express CRY1F protein for resistance to certain lepidopteran insect pests, such as the European corn borer (ECB), and *Sesamia* spp., and to express phosphinothricin-N-acetyltransferase (PAT) protein for tolerance to glufosinate-ammonium herbicide.

The genetic modification in 1507 and relevant information on the insert and the donor organisms has been described in detail in **Part C** of **Section 2** of this notification. A summary of those characteristics relevant to the e.r.a. for the placing on the market of 1507 maize has been included below.

The genetic modification:

The particle acceleration method (high-velocity microprojectiles) (Klein *et al.*, 1987) was used to introduce a purified linear DNA fragment (PHI8999A, 6235 bp; Figure 1) containing the *cry*1F and *pat* coding sequences and the necessary regulatory components into cells from the maize line Hi-II resulting in several maize events, such as event TC1507 or, simply 1507. Several plants were regenerated from these putatively transformed maize cells and those that expressed CRY1F and PAT proteins were selected. One of them was identified as 1507 and was selected for breeding with appropriate inbred lines. This plant and its progeny is referred to as *B.t.* Cry1F maize line 1507, or simply known as 1507 maize. Further details of the transformation are given in Point **C.1.** of **Section 2** of this notification and have not been included here.

Other GM maize lines were also obtained in the same manner, such as *B.t.* Cry1F maize line 1360, or simply 1360 maize. The 1360 maize is of particular relevance to this document as it was selected as an appropriate source of maize expressed CRY1F protein (*i.e.*, representative of similar events, such as 1507 maize) for comparison with microbially-derived CRY1F protein produced in *Pseudomonas fluorescens* in the studies that demonstrated equivalence between maize expressed and microbially-derived CRY1F proteins (Evans, 1998; Annex 25). The intention was to be able to produce sufficient quantity of CRY1F protein from a recombinant microbial source (*i.e.*, microbially-derived CRY1F protein) for a broad range of studies. This was necessary because it is not possible to derive high amounts of adequate CRY1F protein from any maize line transformed with insert PHI8999A, *i.e.* 1507 or 1360 maize.

Information on the vector:

No vector was used for the transformation of 1507 maize. As mentioned above, a linear DNA fragment containing the *cry*1F and *pat* coding sequences together with the necessary regulatory components only was used for transformation by particle acceleration. No additional DNA sequences were used for introducing the insert into 1507 maize.

The insert was obtained from plasmid PHP8999 following digestion of the plasmid DNA with the restriction enzyme *Pme*I. As a result, two linear fragments of DNA were obtained: a 6235 bp fragment, *i.e.* the intended insert containing the *cry*1F and *pat* genes; and a 3269 bp fragment not used in transformation. A detailed description of the organization, size and function of the genetic material present in the 3269 bp fragment is provided in Table 2 of **Section 2** of the notification. The 6235 bp (PHI8999A) fragment was subsequently purified by agarose gel electrophoresis and used in the transformation of 1507 maize.

The results of the detailed molecular characterization of the inserted genetic material in 1507 maize by Glatt in 2000 (see Annex 7) support the conclusion that there is no detectable genetic material from the 3269 bp fragment of plasmid PHP8999 that was not intended for the transformation of 1507 maize. In particular and as expected, the *nptII* gene for kanamycin resistance is not present in 1507 maize, as discussed in detail in Point **C.1.c.**, below.

Information on the donors:

As described above, the insert PHI8999A consisted of a linear DNA fragment of 6235 bp containing the synthetic version of plant optimized and truncated *cry*1F gene from *Bacillus thuringiensis* sbsp. *aizawai* with transcription directed by the ubiquitin promoter *ubi*ZM1(2) from *Zea mays* and with a termination sequence derived from ORF25PolyA from *Agrobacterium tumefaciens* extrachromosomal plasmid pTi15995. The insert also contains the synthetic version of plant optimized phosphinothricin-N-acetyltransferase gene sequence, *pat*, from *Streptomyces viridochromogenes* with transcription directed by CaMV 35S promoter and CaMV 35S terminator, from cauliflower mosaic virus.

A complete description of the size, position, source of donor organism and intended function of the DNA sequences contained in the insert, together with appropriate references, is presented in Table 1 of **Section 2** of this notification.

Bacillus thuringiensis is a Gram-positive soil bacterium that was first discovered in Japan on diseased silkworm, in 1901. At least 176 different *B. thuringiensis* products have been registered as pesticides since 1961 and there have been no confirmed reports of dietary toxicity attributable to their use. Furthermore, there have been no confirmed reports of immediate or delayed toxic or allergenic reactions to humans from exposure to any *B.t.*-based pesticides since their registration (EPA, 1995a). The subspecies *aizawai*, donor of the *cry*1F gene, is commercially used to control wax moth larvae and diamondback moth caterpillar and it has been found to be non-toxic to mammals (Cornell University, 1996; McClintock *et al.*, 1995). The CRY1F protein is the active ingredient in pesticides produced from *B. thuringiensis* sbsp. *aizawai* and it has specific toxicity against certain lepidopteran insect pests (target organisms).

Maize, donor of the ubiquitin promoter *ubi*ZM1(2), has a long history as an agricultural crop and factors affecting genetic stability have been well characterised. Also, maize is continually being modified by breeders to improve its quality and agronomic performance. There are no recognised antinutrients in maize which are considered harmful and worthy of quantification or risk management (Del Valle *et al.*, 1983; White and Pollak, 1995).

Agrobacterium tumefaciens, donor of the ORF25PolyA terminator sequence, is a Gram-negative, non-sporing, rod-shaped bacterium, closely related to *Rhizobium* which forms nitrogen-fixing nodules on clover and other leguminous plants. *Agrobacterium* contains a plasmid (the *Ti* plasmid) with the ability to enter plant cells and insert a portion of its genome into plant chromosomes (Barker, *et al.*, 1983). Normally, *Agrobacterium* is a plant pathogen causing root deformation mainly with sugar beets, pomefruit and viniculture crops. However, the *Ti* plasmid has been altered, *i.e.* disarmed, to make it a useful vector for plant transformation by removing the sequences involved in pathogenicity and inserting sequences necessary for cloning and replication in the laboratory.

Streptomyces viridochromogenes, donor of the *pat* gene, is a common soil bacterium that produces the tripeptide L-phosphinothricyl-L-alanyl-alanine (L-PPT), which was developed as a non-selective herbicide by Hoechst Ag. (Sykes, 1998). The *pat* gene, which confers *S. viridochromogenes* tolerance to the tripeptide, was also identified and characterized in this organism (OECD, 1999; Annex 6).

Cauliflower mosaic virus, donor of the 35S promoter and terminator sequences, is a DNA caulimovirus with a host range restricted primarily to cruciferous plants (ICTV Database, 1998). It has a double stranded DNA genome within which two distinct promoters, producing 19S and 35S transcripts, have been identified (Kay *et al.*, 1987). The 35S promoter and its variants with enhanced transcriptional activity are constitutively active in several plant species that have been genetically modified.

c) **The GMO**

The 1507 maize has been genetically modified to express the proteins CRY1F and phosphinothricin-N-acetyltransferase (PAT). Expression of the CRY1F protein confers season-long resistance against certain lepidopteran pests, such as the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. It is expressed constitutively and when cultivated, provides control against insect pest damage.

Expression of the PAT protein confers tolerance to application of glufosinate-ammonium herbicide. It is expressed constitutively serving as a selectable marker and when cultivated, the maize plant will tolerate field application rates of 1600 g a.i./ha of glufosinate-ammonium without showing any phytotoxicity symptoms. Tolerance to the herbicide provides an alternative tool for weed control management.

No other traits have been introduced or modified as confirmed by the observation of 1507 maize when cultivated in maize growing areas of the USA, Chile, European Union and Central and Eastern Europe (Points **D.4.** and **D.13.** of **Section 2** of this notification).

Information regarding the genetically modified organism has been provided in **Part D** of **Section 2** of this notification. It includes detailed information on:

a) the inserted genetic material in 1507 maize (*i.e.*, size, structure, copy number, location);

b) developmental stages and parts of the plant where the insert is expressed and characteristics of the maize expressed CRY1F and PAT proteins;
c) how the 1507 maize plants differ from the non-GM maize;
d) genetic and phenotypic stability of 1507 maize;
e) whether there is any change to the ability of 1507 maize to transfer genetic material to other organisms;
f) potential toxic, allergenic or other harmful effects of the genetic modification on human health;
g) safety of 1507 maize to animal health;
h) mechanism of interaction between 1507 maize and target organisms;
i) potential changes in interactions of 1507 maize with non-target organisms;
j) potential interactions with the abiotic environment;
k) detection and identification methods for 1507 maize; and,
l) information about previous releases of 1507 maize.

A summary of the information on 1507 maize relevant to this e.r.a. for the placing on the market of 1507 maize has been included below.

The inserted genetic material:

The genetic modification in 1507 maize has been characterised in detail by Southern blot and DNA sequence analyses. The analyses have confirmed that the inserted genetic material is integrated into the nuclear genome of the maize plant and consists of an almost full-length copy of the linear fragment used in the transformation (*i.e.*, 6186 bp from the 6235 bp of insert PHI8999A, containing the *cry*1F and *pat* genes together with the regulatory sequences necessary for their expression). In addition, the plant insert contains the following non-functional fragments:

- one fragment (335 bp) of the *cry*1F gene, with no *ubi*ZM1(2) promoter sequence, and one fragment (15 bp) of the *cry*1F gene, both located at the 5' end of the almost full-length insert;
- two fragments (201 bp and 138 bp long, respectively) of the *pat* gene, without regulatory sequences associated, located at the 5' border and, one fragment (188 bp) of the *pat* gene, located at the 3' border;
- one fragment (118 bp) of the polylinker region and *ubi*ZM1(2) promoter sequence located at the 5' border;
- one fragment (550 bp) of the ORF25PolyA terminator sequence in inverted position located immediately at the 3' end of the almost full-length insert.

The 1507 maize does not contain the *nptII* gene nor any other detectable fragments from the portion of plasmid PHP8999 that was not intended for transformation of 1507 maize. Maize genomic DNA flanking regions at both the 5' and 3' borders of the 1507 maize insert have been sequenced and characterised in detail. Analysis by PCR amplification has confirmed the presence of both maize genomic flanking regions in non-GM Hi-II maize used in the transformation of 1507 maize.

Expression of the insert:

Expression of CRY1F and PAT proteins in a range of tissues from different developmental stages of a typical maize plant was characterized using specific

Enzyme Linked Immunosorbent Assay (ELISA) developed for each protein. The results obtained show that CRY1F protein is expressed throughout the different developmental stages tested, while PAT protein could only be measured at the V9 stage at levels ranging from below the LOD (20 pg/µg total extractable protein) to 136.8 pg/µg total extractable protein in leaves of 1507 maize, and from below the LOD to 38.0 pg/µg total extractable protein in whole plant tissue (V9 stage), but could not be detected in R1 or R4 tissues, senescent plant or mature grain.

The characteristics of the CRY1F and PAT proteins expressed in 1507 maize were examined by Western blot analysis (Alarcon and Marshall, 2000; Annex 8). The CRY1F protein was detected as two bands of approximately 65 and 68 kDa, respectively, and no other bands indicative of a partial CRY1F protein or a fusion protein of greater molecular weight were observed. These two forms of maize expressed CRY1F protein would be expected as a result of limited proteolysis of the ~68 kDa protein by a plant protease with trypsin-like specificity, which results in the ~65 kDa CRY1F form. The only difference between these two forms of maize expressed CRY1F protein is the presence or absence of amino acids 1 to 27 from the N-terminus of the CRY1F protein, which are absent from the ~65 kDa CRY1F form. The biochemical structure of these two forms is comparable as shown by their equivalent immunoreactivity in ELISA and Western blot analyses (Evans, 1998; Alarcon and Marshall, 2000; Annexes 25 and 8, respectively). In addition, the biological activities of the two forms of maize expressed CRY1F protein will be identical once they are ingested by the target pest, as proteolytical processing in the alkaline conditions found in the gut of target insect pests will result in comparable forms of CRY1F protein (such as the 65 kDa form). A similar post-translational processing on the removal of amino acids 1-27 from the N-terminus of the pro-toxin also occurs for other CRY proteins, such as CRY1A (Schnepf *et al.*, 1998).

The PAT protein is known to be a homodimer of approximately 43 kDa in its native form, and it is comprised of two components of approximately 22 kDa (OECD, 1999; Annex 6). The results of the Western blot analysis of 1507 maize confirmed the presence of the ~22 kDa PAT monomeric form and of the ~43 kDa PAT homodimer in leaf tissue. No other bands indicative of a partial PAT protein or fusion protein of greater molecular weight were observed in 1507 maize.

Differences between 1507 maize and non-GM maize:

The genetic modification in 1507 maize results in expression of CRY1F protein conferring resistance to certain lepidopteran insect pests, and PAT protein conferring tolerance to glufosinate-ammonium herbicide. No other traits have been introduced or modified in 1507 maize (Point **D.4.** of **Section 2** of this notification).

The efficacy of 1507 maize hybrids against a range of insect pests has been determined and compared with conventional maize controls under field conditions. The insects evaluated were European corn borer (*Ostrinia nubilalis*), fall armyworm (*Spodoptera frugiperda*), corn earworm (*Helicoverpa zea*), southwestern corn borer (*Diatraea grandiosella*), black cutworm (*Agrotis ipsilon*), and sugarcane borer (*Diatraea saccharalis*). Further tests have been carried out which have confirmed the

effectiveness of 1507 maize against *Sesamia* spp. infestation (Castañera, 2001; Annex 16).

A separate field study was carried out in 2000 in France to compare 1507 maize (without application of insecticide) and non-GM maize with application of a synthetic insecticide (Karate Xpress, active ingredient lambda-cyhalothrin) commonly used to control European corn borer infestation (Vernier, *et al.*, 2001b; Annex 34). The 1507 maize showed significant efficacy to control both European corn borer and pink stalk borer (*Sesamia nonagrioides*) and providing better protection from both pests than traditional insecticide applications.

In summary, the results show that 1507 maize provides much improved control for lepidopteran insects pests in comparison to application of insecticides to conventional (non-GM) maize.

Additional field trials were carried out in Spain in 2002 for agronomic purposes at three separate locations in the region of Aragón. In summary, a comparison of the agronomic characteristics of 1507 maize and non-GM maize with comparable genetic background cultivated under Spanish conditions confirmed that there were no unexpected adverse differences. In fact and taking into account the significant target pest pressure from ECB and *Sesamia* spp. that occurred in Aragón (Spain) in 2002, the agronomic differences observed in terms of less insect damage, higher yield, higher plant height and ear, and less stalk lodging of 1507 maize plants result from resistance against attack from target insect pests conferred by the expression of CRY1F protein, as intended by the genetic modification.

The active ingredient in glufosinate-ammonium herbicide is L-phosphinothricin (L-PPT). L-PPT binds to glutamine synthetase in plants preventing the detoxification of excess ammonia resulting in plant death. The activity of the PAT protein (phosphinothricin-N-acetyltransferase) is specific to catalysing the conversion of L-PPT to N-acetyl-L-PPT. Expression of PAT protein in 1507 maize allows the detoxification of ammonia to continue thereby conferring tolerance to the herbicide glufosinate-ammonium. Field trials have shown that 1507 maize is tolerant to field application rates of 1600 g a.i./ha of glufosinate-ammonium herbicide without showing any phytotoxicity symptoms. This rate is equivalent to four times the recommended field application rate of the glufosinate-ammonium herbicide.

Agronomic data was obtained from field trials of 1507 maize carried out at different locations across key maize growing regions in the USA in 1999; in France, Italy and Bulgaria in 2000, and in Spain in 2002. The results support the conclusion that there are no unexpected agronomic differences between 1507 maize and non-GM maize, and that 1507 maize has no altered survival, multiplication or dissemination characteristics.

Genetic and phenotypic stability of 1507 maize:

Analysis of the Mendelian segregation of the inserted traits has provided evidence of the genetic stability of the inserted genetic material in 1507 maize (Point **D.5.** of **Section 2** of this notification). The *cry*1F and *pat* genes are integrated in the genome of the plant and inherited as Mendelian dominant genes.

Change to the ability of 1507 maize to transfer genetic material to other organisms:

There are no sequences in the genetic material inserted into 1507 maize that could potentially be involved in transfer of genetic material between maize and other organisms. As a result, there is no change in the ability of 1507 maize to transfer genetic material to other organisms (Point **D.6.** of **Section 2** of this notification).

Potential toxic, allergenic or other harmful effects of the genetic modification on human health:

The results obtained from the toxicological studies confirm that the genetic modification in 1507 maize does not introduce any new compounds known to be toxic, allergenic or harmful to human or animal health (Point **D.7.** of **Section 2** of this notification). A summary of the results has been included below.

As mentioned in Point **C.1.b.** above, in order to have sufficient amounts of purified CRY1F protein for the multiple studies required to assess the safety of maize expressed CRY1F protein, a form of the protein with equivalent biochemical structure and biological activity to the structure and activity of maize expressed CRY1F was produced in *Pseudomonas fluorescens* (Evans, 1998; Annex 25). The complete description of the sequence and key characteristics of microbially-derived CRY1F protein have been included in Point **D.7.** of **Section 2** of this notification.

Toxicity:

An acute oral toxicity study with microbially-derived CRY1F protein have been carried out with mice. No mortality, toxicity or adverse clinical signs were observed at the highest dose tested of 5050 mg of test material per kg of body weight which was equivalent to 576 mg of pure CRY1F protein per kg of body weight (Kuhn, 1998). The relatively high dose tested in this study did not give rise to any toxicity and therefore the acute LD_{50} for CRY1F protein could not be determined other than estimated to be higher than 576 mg CRY1F per kg of body weight.

The safety in terms of toxicity for the PAT protein has already been determined in detail during the assessment of glufosinate-ammonium tolerant maize (EPA, 1995b; EPA, 1997; Canadian Food Inspection Agency, 1998; SCP, 1998; OECD, 1999; Annex 6). A toxicity study consisting of feeding rats with the PAT protein (0, 5000 and 50000 mg/kg body weight) has been carried out (Pfister *et al.*, 1996; Health Canada, 1997) and the results showed the absence of any adverse treatment-related clinical signs. In addition, an acute oral toxicity study consisting of feeding mice with 6000 mg test material per kg body weight containing approximately 5000 mg PAT per kg body weight has been carried out (Brooks, 2000). There were no adverse clinical

signs and the LD_{50} for PAT protein could not be determined other than estimated to be higher than 5000 mg PAT per kg body weight.

In addition, a poultry feeding study over a period of 42 days has also been carried out with grain from 1507 maize and non-GM control maize with comparable genetics (Point **D.7.** of **Section 2** of this notification and Annex 5). The mortality, body weight gain and feed conversion of the chickens fed with 1507 maize were compared to chickens fed a standard diet containing yellow dent maize. No statistically significant differences were observed on mortality, body weight gain or feed conversion between chickens fed a diet containing grain from 1507 maize or any of the other diets.

A thirteen-week (90-day) oral toxicity feeding study in rats has been carried out with 1507 maize grain in order to confirm the absence of toxicity of the proteins CRY1F and PAT expressed in 1507 maize grain (Annex 39). The results obtained confirm that no toxicologically significant diet-related differences were observed among the groups fed with any of the different diets with respect to body weight, body weight gain, food consumption, food efficiency, clinical signs of toxicity, ophthalmological observations, neurobehavioral assessments, clinical pathology (hematology, clinical chemistry, coagulation, or urinalysis parameters), organ weights, and gross or microscopic pathology.

Allergenicity:

A detailed assessment of the allergenic potential of the CRY1F and PAT proteins has been made in Point **D.7.** of **Section 2** of this notification following the recommendations from FAO/WHO (2000), and according to the decision-tree of Metcalfe *et al.* (1996) for the assessment of the allergenicity potential of genetically modified crop plants.

The conclusions obtained from the assessment for allergenicity, together with other relevant criteria, confirm that the *cry*1F and *pat* genes introduced into 1507 maize do not encode for any known allergens and that the CRY1F and PAT proteins, as expressed in 1507 maize, show a very low probability of being allergenic. A summary of the main points has been included below:

a) There is no history of allergenicity from *Bacillus thuringiensis* sbsp. *aizawai* and *Streptomyces viridochromogenes*, donor organisms for the *cry*1F and *pat* genes, respectively (EPA, 1995a; McClintock *et al.*, 1995; EPA, 1996; OECD, 1999);
b) Neither the CRY1F nor the PAT proteins share immunologically significant amino acid sequences with known allergens (Meyer, 1999; OECD, 1999);
c) The CRY1F and PAT proteins are rapidly digested under simulated gastric fluid conditions (Evans, 1998; OECD, 1999; Annexes 25 and 6, respectively);
d) The absence of post-translational glycosylation of maize expressed CRY1F and PAT proteins (Evans, 1998; OECD, 1999; Annexes 25 and 6, respectively);

e) The CRY1F and PAT proteins are susceptible to heating (Evans, 1998; Herman, 2000; OECD, 1999; Annexes 25, 23 and 6, respectively);
f) The molecular weight of maize expressed CRY1F protein is 65 to 68 kDa, in the range of other non-allergenic CRY proteins;
g) The biological activities of the CRY1F and PAT proteins are very specific and do not indicate any concern regarding allergenicity. The CRY1F protein shows specific toxicity against certain lepidopteran insect pests, and the PAT protein allows the detoxification of ammonia by the specific conversion of L-PPT to N-acetyl-L-PPT; and,
h) The expression level of CRY1F protein in 1507 maize plant tissue and grain is relatively low, whereas the PAT protein is only detectable at low levels in vegetative leaf tissue.

Safety of 1507 maize to animal health:

A detailed safety evaluation concerning possible feed applications of 1507 maize and feed products derived from 1507 maize (processed and non-processed) has been carried out and is attached as Annex 1.

The conclusions obtained confirm that feed products from 1507 maize are substantially equivalent to, nutritionally equivalent to, and as safe as, feed products derived from commercially available (non-GM) maize. This is based on the compositional analyses comprising protein, fiber, carbohydrates, ash, minerals, fatty acids, amino acids, vitamins, secondary metabolites and anti-nutrients in forage and/or grain samples from 1507 maize; the nutritional equivalence shown in a poultry feeding study; and, the detailed safety evaluation of the expressed CRY1F and PAT proteins as intended by the genetic modification in 1507 maize.

The feed products obtained from 1507 maize are comparable to those obtained from non-GM maize and there are no known toxic, allergenic or other harmful effects to animal health arising from the genetic modification.

Mechanism of interaction between 1507 maize and target organisms:

The mechanism of interaction between maize expressed CRY1F protein and target organisms has been described in detail in Point **D.9.** of **Section 2** of this notification and can be summarized as follows:

Maize expressed CRY1F protein consists of residues 1 to 605 of the native CRY1F sequence from *B. thuringiensis* sbsp. *aizawai*, with a single and conservative amino acid substitution (F to L at position 604), which has a predicted molecular weight of 68204.56 Da (ExPASy Server) and corresponds to the band of protein of approximately 68 kDa observed in the Western blot analyses of 1507 maize (Alarcon and Marshall, 2000; Annex 8). A fraction of maize expressed CRY1F protein (68 kDa) is post-translationally digested by maize trypsin-like proteases resulting in the removal of amino acids 1 to 27 (inclusive) from the CRY1F sequence. This second form of the CRY1F protein corresponds to the band of protein of approximately 65 kDa also observed in the Western blot analyses of 1507 maize by Alarcon and Marshall (2000) (Annex 8). This mechanism of production of activated toxins that are typically 65-70 kDa in size is similar to that of other CRY proteins.

Upon ingestion of 1507 maize tissue by susceptible insects (target pests) the two forms of maize expressed CRY1F protein will reach the alkaline conditions of the insect gut where they may be processed further by trypsin-like proteases before binding to specific receptors on the apical microvilli of epithelial midgut cells of the insect and undergo a conformational change that allows insertion into the membrane of the cell. Toxin oligomerization will then occur with formation of pores in the membrane of the midgut cells of the insect causing osmotic cell lysis leading to insect death.

Potential changes in the interactions of 1507 maize with non-target organisms:

As described in detail in Point **D.10.** of **Section 2** of this notification, the specific biological activity of CRY1F and PAT proteins expressed in 1507 maize together with the absence of toxicity of CRY1F protein to non-target and beneficial organisms provides strong evidence for the absence of any significant toxicity to non-target organisms which may arise from exposure to 1507 maize. Therefore, there are no potential changes in the interactions of 1507 maize with non-target organisms resulting from the genetic modification.

The results from specific studies on a range of non-target organisms are summarized below (microbially-derived CRY1F protein was used in some of these studies after obtaining confirmation of equivalence to the maize expressed CRY1F protein (Evans, 1998; Annex 25)).

Non-target arthropods

A series of dietary toxicity studies were carried out on representative non-target insects. These included the green lacewing larvae (*Chrysoperla carnea*); the ladybird beetle (*Hippodamia convergens*); and, the beneficial parasitic Hymenoptera *Nasonia vitripennis*. No mortality or signs of toxicity were observed on the green lacewing larvae (Hoxter *et al.*, 1999a; Annex 26), the ladybird beetle (Hoxter *et al.*, 1999b; Annex 27), or the beneficial parasitic *N. vitripennis* (Hoxter *et al.*, 1999c: Annex 28). As a result, the LC_{50} values for each of the non-target insects could not be established and therefore estimated to be higher than 480 ppm for the green lacewing larvae and the ladybird beetle (*i.e.* up to 30 times the concentration of CRY1F protein present in pollen from 1507 maize) and higher than 320 ppm for the beneficial parasitic Hymenoptera *N. vitripennis* (*i.e.* up to 20 times the concentration of CRY1F protein present in pollen from 1507 maize).

A toxicity study with the monarch butterfly (*Danaus plexippus*) demonstrated no toxicity of CRY1F protein to this non-target lepidopteran (Bystrak, 2000). The LC_{50} value for CRY1F protein to monarch butterfly neonates could not be determined because there was no mortality at the highest dose tested (10000 ng/ml diet), which was the highest dose that could be physically incorporated into the diet. Further tests using higher concentration of microbially-derived CRY1F protein have confirmed that the LC_{50} for CRY1F to monarch butterfly neonates could not be determined and therefore estimated to be higher than 30 ppm (Blair Siegfried, personal communication).

In addition, the level of beneficial arthropods present in field plots of 1507 maize were compared to those in field plots of non-GM maize with comparable genetics (Higgins, 1999; Annex 30). The numbers of adult and larval lady beetles (*Cycloneda munda* and *Coleomegilla maculata*), insidious flower bugs (ssp. *Orius insidiosus*) assassin bugs (Family: Reduviidae), damsel bugs (Family: Nabidae), brown lacewings (Family: Hemerobiidae), green lacewings (*Chrysoperla plorabunda*), predatory beetles (Family: Carabidae) and parasitic Hymenoptera (Family: Ichneumonidae and Brachonidae), damsel or dragonflies (Order: Odonata), and spiders were assessed either visually and/or with traps. The results demonstrated that expression of CRY1F protein in 1507 maize had no effect on the presence of the beneficial arthropods observed.

A faunistic field study has been carried out in 2000 in France to study the complex tritrophic interactions in the 1507 maize ecosystem compared to the non-target effects observed after application of a synthetic insecticide (Karate Xpress, active ingredient lambda-cyhalothrin) commonly used to control European corn borer infestation (Vernier *et al.*, 2001b; Annex 34). The results clearly showed that while the Karate Xpress treatment significantly reduced the population of non-target arthropods such as thrips, *Orius* sp. and leafhoppers, there were no adverse effects of 1507 maize in the population of non-target arthropods.

Furthermore, a field survey of non-target arthropods associated with 1507 maize in the Spanish maize system was conducted at two locations in 2002 (Annex 33). The results obtained do not highlight any potential differences in how beneficial arthropods use conventional and 1507 maize supporting previous risk assessment studies of non-target organisms in 1507 maize where favorable conclusions have been reached (Higgins 1999, Vernier *et al.* 2001a; Annexes 30 and 36, respectively).

In addition, a detailed non-target exposure and risk assessment for the placing on the market of 1507 maize has been carried out by Wolt and Conlan (2001) and is attached as Annex 35. The conclusions obtained from this detailed study confirm that there is no significant risk for any adverse effects on non-target organisms, and in particular there will be no significant adverse effects on sensitive non-target lepidopteran species from exposure to cultivated 1507 maize.

Honey bees

No effects were observed on larval survival nor adult behaviour in honey bees (*Apis mellifera*) (Maggi, 1999; Annex 31). A single dose of 2 mg of pollen from 1507 maize or of 5.6 µg of microbially-derived CRY1F protein suspended in a 30% sucrose solution was administered to each cell. The results indicate that the CRY1F protein does not adversely affect either survival of honey bee larvae nor their emergence.

Terrestrial organisms

Microbially-derived CRY1F protein shows no toxicity to earthworms (*Eisenia foetida*) at a concentration equivalent of up to 100 times the incorporation of senescent 1507 plants into the top 15 cm of soil (at a rate of 62000 plants per hectare) (Hoxter *et al.*, 1999d; Annex 29). Therefore, the LC_{50} could not be established and therefore estimated to be higher than 1.7 mg of CRY1F per kg of dry soil.

A laboratory study to determine the chronic effects of CRY1F protein on survival and reproduction of the soil dwelling invertebrate collembola (*Folsomia candida*), which plays a major role in soil ecosystems due to their feeding on decaying plant materials, has also been carried out (Halliday, 1998a). The results indicated that concentrations representing estimated exposure rates that are 1560-, 388- and 79-fold higher than those that would be found in the field did not cause any significant adverse effects on collembola after feeding on these diets for 28 days.

Wildlife birds

Grain from 1507 maize was ground and fed to juvenile northern bobwhite quail (*Colinus virginianus*) in the diet for 5 days (Gallagher *et al.*, 1999). The results showed that there were no adverse effects and the dietary LC_{50} value could not be established and therefore estimated to be higher than 100000 mg of 1507 maize grain per kg of diet.

Aquatic organisms

A 48-hour static-renewal acute toxicity test with the cladoceran aquatic invertebrate *Daphnia magna* was conducted using the microbially derived CRY1F protein and pollen from 1507 maize (Drottar and Krueger, 1999). The 48-hour EC_{50} value for *Daphnia magna* exposed to CRY1F protein could not be established and therefore estimated to be higher than 100 mg CRY1F per liter. Pollen from 1507 maize did not cause any mortality and the EC_{50} value could not be established and therefore estimated to be higher than 100 mg of 1507 maize pollen per liter.

A fish toxicity test was considered not necessary because ELISA analyses and bioassays demonstrated that CRY1F was not detectable nor biologically active in the fish diet prepared with 1507 maize grain containing the maximum concentration of maize found in fish diets (35 to 40%) (Mayes, 1999; Annex 24). Commercial manufacture of fish diets involves a heating step that would degrade the CRY1F protein.

Potential interactions with the abiotic environment

As discussed in Point **D.11.** of **Section 2** of this notification, expression of the CRY1F and PAT proteins in 1507 maize does not alter the natural interactions of maize plants with the abiotic environment. The very limited persistence of microbially-derived CRY1F protein in the soil environment (DT_{50} = 3.13 days; Halliday, 1998b; Annex 32) coupled with the natural ubiquity of the *cry*1F and *pat* genes in the soil environment and the absence of adverse effects on soil biota means negligible possibility for adverse interactions with the abiotic environment and no adverse effects on the biogeochemical cycles.

Detection and identification methods for 1507 maize:

The 1507 maize can be detected and identified by placing small amounts of glufosinate-ammonium herbicide on leaves of maize plants (Point **D.12.** of **Section 2** of this notification). Maize plants with expression of PAT protein will be those with

leaves that do not show any necrosis at point of herbicide application. Alternatively, maize plants can be sprayed with glufosinate-ammonium herbicide, and those that survive will be expressing PAT protein.

ELISA can also be used to detect the expression of the CRY1F and PAT proteins. Additionally, an insect bioassay with sensitive lepidopteran insect species can be used to identify maize plants expressing the CRY1F protein.

A PCR detection method unique for 1507 maize has been developed which can also be used to confirm the molecular identity of 1507 maize (Annex 15).

Information about previous releases of 1507 maize:

Previous releases of 1507 maize into the environment have taken place in the EU, in accordance with Part B of Directive 90/220/EEC, and also outside the EU, as described in detail in Point **D.13.** of **Section 2** of this notification. The results obtained from these previous releases show no adverse effects on human health and the environment from 1507 maize.

d) The intended release or use including its scale

The 1507 maize is intended to be used as any other commercially available maize for all possible downstream uses towards animal feed or any other application arising from import or cultivation. An application for food use of 1507 maize has been submitted on 15 February 2001 to the Competent Authority of The Netherlands in accordance with Regulation (EC) No. 258/97.

As mentioned in Point **A.5.** of **Section 3** of this notification, maize is widely cultivated in a variety of agricultural environments of the EU, with strong variations at a regional level throughout the Community. The largest producer is France (43%), followed by Italy (25%), Spain (11%) and Germany (8%). Grain and derived products from 1507 maize are expected to be part of this production.

e) The potential receiving environment

The 1507 maize will be cultivated in agricultural environments throughout the EU as any other commercially available maize, as mentioned in Point **C.1.d.** above.

f) The interaction between the intended release or use including its scale and the potential receiving environment

The interaction between the intended use of 1507 maize including its scale and the receiving environment is not expected to cause any specific effects different from those of any other commercially available maize, with the sole exception of the potential development of resistance in the target insect pest population during cultivation. This limited potential risk will be appropriately managed by the proposed insect resistance management (IRM) plan (Annex 37) developed in the context of product stewardship and in conjunction with the monitoring plan developed in accordance with Annex VII of Directive 2001/18/EC (**Section 5** of this notification).

g) **Information from releases of similar organisms and organisms with similar traits and their interaction with similar environments**

So far the following similar GM organisms have received approval in the European Union under Directive 90/220/EEC:

Product	Trait	Use	Notification
Bt-176	Expressing CRY1Ab protein for resistance against certain lepidopteran insect pests	Import, cultivation	C/F/94/11/03
T25	Expressing PAT protein for tolerance against glufosinate-ammonium herbicide	Import, cultivation	C/F/95/12/07
MON810	Expressing CRY1Ab protein for resistance against certain lepidopteran insect pests	Import, cultivation	C/F/95/12/02
Bt-11	Expressing CRY1Ab protein for resistance against certain lepidopteran insect pests and PAT protein for tolerance against glufosinate-ammonium herbicide	Import	C/GB/96/M4/1

To date no adverse effects arising from the interaction of these GM organisms with the EU environment have been recorded or published. In particular, Bt-176 maize varieties have been cultivated over 20,000-25,000 ha each year since 1998 in Spain. This limited cultivation has already shown some important benefits such as absence of application of insecticides used to control ECB, reduced exposure to insecticides by farmers, net savings in energy use and improved yields. In fact, reduction in the use of insecticides to control ECB has resulted in a perceived positive impact to the environment in those areas with high incidence of ECB attack (Brookes, 2002).

C.2. STEPS IN THE ANALYSIS OF ENVIRONMENTAL RISK ASSESSMENT

1. Step 1: Identification of characteristics which may cause adverse effects

1.1. Characteristics of the GMO linked to the genetic modification

The characteristics of 1507 maize linked to the genetic modification have been described in detail on **Part C** of **Section 2** of this notification and summarized in Point **C.1.b.**, above. Two new genetic traits are introduced in 1507 maize: the plant optimized and truncated *cry*1F gene and the plant optimized *pat* gene. No other new traits have been introduced into 1507 maize, as confirmed by the molecular characterization of the sequences actually inserted (Point **D.2.a.** of **Section 2**), expression of the insert (Point **D.3.** of **Section 2**), agronomic performance (Point **D.4.** of **Section 2**), and comparable composition to other conventional maize (Annex 1).

Resistance of 1507 maize to insect pests as conferred by the *cry*1F gene:

The *cry*1F gene is expressed constitutively by the *ubi*ZM1(2) promoter in 1507 maize. Expression of CRY1F protein provides season-long control against lepidopteran insect pest damage to the maize plant during cultivation. Specifically, the expression of CRY1F protein confers resistance against the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. It is also highly effective against corn earworm (*Helicoverpa zea*), fall armyworm (*Spodoptera frugiperda*), black cutworm (*Agrotis ipsilon*) and southwestern corn borer (*Diatraea grandiosella*) (Point **D.4.** of **Section 2** of this notification).

Tolerance of 1507 maize to glufosinate-ammonium herbicide as conferred by the *pat* gene:

The *pat* gene is expressed constitutively by the CaMV 35S promoter in 1507 maize. Expression of PAT protein confers tolerance to application of glufosinate-ammonium herbicide. Field trials show that 1507 maize will tolerate field application rates of 1600 g a.i./ha of glufosinate-ammonium herbicide without showing any phytotoxicity symptoms (four times the recommended rate). Tolerance to glufosinate-ammonium herbicide provides for an alternative in weed management.

The *pat* gene and expression of PAT protein in maize have been the subject of a previous safety evaluation in the EU. Expression of *pat* gene in T25 maize was notified in the EU by AgrEvo(Aventis), now called Bayer CropScience (EU Notification Number C/F/95/12/07) and was granted consent on the 3rd August 1998 by the French Competent Authority for placing on the EU market in accordance with Commission Decision under Directive 90/220/EEC (Commission Decision of 22 April 1998 (98/293/EC)). The EU decision for approval was adopted after carrying out comprehensive evaluation for human health and environmental safety on the PAT protein as expressed in the T25 maize: the EC Scientific Committee on Plants concluded that there was no evidence to indicate that the use of T25 maize as any other maize was likely to cause adverse effects on human or animal health and the environment (SCP, 1998).

1.2. Potential adverse effects of the GMO(s)

a) Disease to humans including toxic or allergenic effects

Maize is extensively cultivated and has a history of safe use: it is not an organism which causes disease to humans. In fact, maize or derived products of maize are not considered to be harmful. The insertion of the *cry*1F and *pat* genes and the expression of the CRY1F and PAT proteins in 1507 maize does not introduce any new compounds known to cause disease, toxicity or allergenicity to humans. As described in detail in Point **D.7.** of **Section 2** of this notification and Point **C.1.c.**, above, this conclusion is based on the following evidence:

i) The CRY1F protein is specific against certain lepidopteran insect pests and is non-toxic against mice (LD_{50} > 576 mg/kg bw).

ii) The PAT protein is enzymatically active but has high substrate specificity for L-PPT and is non-toxic against rats (LD_{50} > 5000 mg/kg bw) or mice (LD_{50} > 5000 mg/kg bw).

iii) The donor organisms for the *cry*1F and *pat* genes, *B. thuringiensis* sbsp. *aizawai* and *S. viridochromogenes*, respectively, are common soil bacteria and do not have a history of causing toxicity or allergenicity to humans.

iv) Neither CRY1F nor PAT proteins show any significant sequence homology to known allergenic proteins.

v) The biochemical characteristics of CRY1F and PAT proteins are indicative of absence of any allergenic potential (e.g. rapid degradation in simulated gastric fluid (SGF); lack of post-translational glycosylation; and, susceptibility to heating); and, the CRY1F protein shares comparable proteolytic characteristics in SGF and simulated intestinal fluid (SIF) systems with other CRY proteins which are not considered to share the characteristics of known food allergens (e.g., CRY1A(b) and CRY3A).

vi) The contents of CRY1F and PAT proteins per total extractable protein in 1507 maize are very low.

vii) No toxicologically significant diet-related differences were observed in a thirteen-week (90-day) oral toxicity feeding study in rats with respect to body weight, body weight gain, food consumption, food efficiency, clinical signs of toxicity, ophthalmological observations, neurobehavioral assessments, clinical pathology (hematology, clinical chemistry, coagulation, or urinalysis parameters), organ weights, and gross or microscopic pathology.

As a result, any potential adverse effects of 1507 maize on human health are comparable to commercially available (non-GM) maize and have not been significantly altered by the insertion of the *cry*1F and *pat* genes resulting in expression of CRY1F and PAT proteins.

b) Disease to animals and plants including toxic, and where appropriate, allergenic effects

Maize is not considered to have any toxic effects on animals. It has a long history of safe use as animal feed and does not cause any disease. In fact, maize is popular as animal feed because it does not contain significant levels of anti-nutrient compounds or endogenous toxins.

Insertion of the *cry*1F and *pat* genes and expression of the CRY1F and PAT proteins in 1507 maize does not alter safety of maize to animal health. Neither CRY1F nor PAT proteins cause any potential adverse effects on animal health and they are non-toxic to animals and wildlife, as described in detail in Points **D.7.** and **D.11.** of **Section 2** of the notification and summarized in Point **C.1.c.**, above. In addition, a poultry feeding study with grain from 1507 maize has confirmed the nutritional equivalence between 1507 maize grain and grain from commercially available (non-GM) maize.

There is no evidence of either CRY1F or PAT proteins conferring disease to plants. The results obtained from previous releases of 1507 maize indicate that there are no unexpected adverse effects on the health of 1507 maize plants (Point **D.13.** of **Section 2** of this notification).

c) Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of each of these populations

Maize interacts with other organisms in the environment including insects, birds and mammals. It is also susceptible to a range of fungal diseases and insect pests, as well as competition from surrounding weeds.

Effects on populations of non-target organisms:

As described in detail in Points **D.4.** and **D.9.** of **Section 2** of this notification and in Point **C.1.c.**, above, the specificity of the biological activity of CRY1F protein against certain lepidopteran pests and the high substrate specificity of PAT protein for L-PPT provides strong support for the absence of any significant adverse effects on the dynamics of populations in the receiving environment and the genetic diversity of each of these populations.

In addition, microbially-derived CRY1F protein shows a very limited persistence in the soil environment (DT_{50} = 3.13 days; Halliday, 1998b; Annex 32), which, together with the natural ubiquity of the *cry*1F and *pat* genes in the soil environment and the absence of adverse effects on soil biota, means negligible possibility for effects on populations of soil dwelling organisms.

Furthermore, a detailed non-target exposure and risk assessment for the placing on the market of 1507 maize has been elaborated by Wolt and Conlan (2001) and is attached as Annex 35. The conclusions obtained from this detailed study confirm that there is no significant risk for any adverse effects on non-target organisms, and in particular on sensitive non-target lepidopteran species, arising from exposure to 1507 maize. In fact, the simulations performed confirm that only very limited effects on a

hypothesized extremely sensitive lepidopteran species feeding within a 1 m radius from the edge of the maize field may take place. The common and unprotected lepidopteran species are widely distributed in the EU across various habitats and they will not be significantly affected from exposure to pollen from 1507 maize. Also, protected species are unlikely to be associated with arable land used for maize cultivation due to habitat preferences such as marshy, arid, or alpine (> 1000 m elevation) environments (Wolt and Conlan, 2001; Annex 35).

In conclusion, negligible effects are expected on the dynamics of populations of non-target organisms in the receiving environment and the genetic diversity of each of these populations.

Effects on populations of target organisms:

As described in **Section 2** of this notification and in Point **C.1.c.** above, cultivation of 1507 maize provides growers with a highly effective and environmentally beneficial tool to control certain lepidopteran insect pests (target organisms), such as the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. This specific effect on certain target organisms is intended by the genetic modification in 1507 maize and therefore is not considered as an adverse effect on populations of target organisms.

However, this benefit would be reduced if the target insect pests develop resistance to CRY1F protein as expressed in 1507 maize during cultivation (Point **D.4.**, below). In the light of current thinking and existing experience with Bt maize products, a detailed proposal for an insect resistance management (IRM) plan has been developed in the context of product stewardship and is attached to this notification as Annex 37. The IRM plan will be applied in conjunction with the monitoring plan developed in accordance with Annex VII of Directive 2001/18/EC (**Section 5** of this notification).

d) Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors

There have been no signs observed of any altered susceptibility of 1507 maize to pathogens from previous field trials with 1507 maize. The assessment of the agronomic characteristics of 1507 maize (Point **D.4.** of **Section 2** of this notification) confirms that it is comparable to other commercially available (non-GM) maize with the exception of the traits introduced by the genetic modification: CRY1F protein expression conferring resistance to certain lepidopteran pests and expression of PAT protein conferring tolerance to glufosinate-ammonium herbicide. Therefore, no adverse effects are expected to human health or the environment that may be caused by alteration of maize susceptibility to pathogens.

e) Compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments

The genetic modification in 1507 maize does not compromise prophylactic or therapeutical medical, veterinary, or plant protection treatments. As described in Point **D.2.** of **Section 2** of this notification and Point **C.1.c** above, no genetic material coding for genes conferring resistance to antibiotics used in human or veterinary

medicine has been inserted in 1507 maize. In particular, the *nptII* gene, conferring resistance to kanamycin was not part of insert PHI8999A used to transform 1507 maize and it is not present in the genome of 1507 maize, as confirmed by Southern blot analysis (Glatt, 2000; Annex 7).

f) Effects on biogeochemistry (biogeochemical cycles), particularly carbon and nitrogen recycling through changes in soil decomposition of organic material

As discussed in detail in **Point D.1** of **Section 2** of this notification, the toxicity of CRY1F protein is specific to certain target lepidopteran insect pests. In particular and as discussed in detail in **Point D.10** of **Section 2** of this notification, no adverse effects of 1507 maize have been observed on non-target and beneficial organisms, and in particular on soil dwelling organisms, such as earthworms (*Eisenia foetida*) and collembola (*Folsomia candida*). Furthermore, the very limited persistence of the CRY1F protein in the soil environment, the natural ubiquity of the *cry*1F and *pat* genes in the soil environment, and the specific biochemical activity of PAT protein confirm that the genetic modification in 1507 maize will not cause any adverse effects on biogeochemical cycles resulting from any potential direct and indirect interaction of 1507 maize and target and non-target organisms in the vicinity of 1507 maize.

g) Other potential adverse effects

Adverse effects may occur directly or indirectly through mechanisms which may include:

- The spread of the GMO in the environment
- The transfer of the inserted genetic material to other organisms, or the same organism whether genetically modified or not
- Phenotypic and genetic instability
- Interactions with other organisms
- Changes in management, including, where applicable, in agricultural practices

An evaluation to identify any potential adverse effects on human health or the environment that may occur through these mechanisms has been carried out and the results obtained are presented below.

<u>The spread of the GMO in the environment:</u>

Although cultivation of maize in the EU has taken place for five centuries, starting in Spain, maize has not spread in the EU environment. This is due to the fact that maize does not show any weedy characteristics and is not persistent, and that production of maize in the EU requires the extensive application of agricultural practices.

The genetic modification in 1507 maize does not alter the agronomic characteristics of the plant, as discussed in detail in Point **C.4.** of **Section 2** of this notification. In particular, the capacity of 1507 maize to spread in the EU environment is equivalent to that of any other commercially available (non-GM) maize, *i.e.* null. Therefore, no adverse effects will occur through this mechanism.

The transfer of the inserted genetic material to other organisms, or the same organism whether genetically modified or not:

There are no sexually compatible wild or weedy relatives of *Zea mays* in Europe and therefore outcrossing or transfer of the inserted genetic material to other organisms will not occur. In addition, none of the sequences introduced into 1507 maize are involved in transfer of genetic material between organisms (Point **C.1.c.**, Table 1).

Any potential transfer of the inserted genetic material will be restricted to other maize plants grown in the proximity of 1507 maize, as dispersal of the relatively heavy maize pollen is limited. In any case and based on the studies described in this notification, no adverse effects will occur as CRY1F and PAT proteins are considered to have no adverse effects to human and animal health or the environment.

Phenotypic and genetic instability:

As discussed in detail in Point **D.5.** of **Section 2** of this notification and summarized above (Point **C.1.c.**), 1507 maize is phenotypically and genetically stable.

The traits introduced by the genetic modification, expression of CRY1F protein expression conferring resistance to certain lepidopteran pests and expression of PAT protein conferring tolerance to glufosinate-ammonium herbicide, have been shown to be stable through multiple releases in different agricultural environments and seasons. Also, the genetic material inserted in 1507 maize is integrated in the genome of the plant and is inherited as a dominant Mendelian gene. The characteristics of the CRY1F and PAT proteins expressed in 1507 maize are as expected from the genetic modification and their expression levels have been confirmed to be stable and within normal variation ranges.

In conclusion, there are no adverse effects that may occur through phenotypic or genetic instability of 1507 maize.

Interactions with other organisms:

Please refer to Point **C.2.1.2.c.**, "Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of each of these populations", above.

Changes in management, including, where applicable, in agricultural practices:

Cultivation of 1507 maize expressing CRY1F protein provides growers with a highly effective and environmentally beneficial tool to control certain lepidopteran insect pests, such as the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. However, this benefit would be reduced if the target insect pests develop resistance to CRY1F protein as expressed in 1507 maize during cultivation (Point **D.4.**, below). In the light of current thinking and existing experience with cultivation of Bt maize products, a detailed proposal for an insect resistance management (IRM) plan has been developed in the context of product stewardship and is attached to this document (Annex 37). The IRM plan will be applied in conjunction with the monitoring plan

developed in accordance with Annex VII of Directive 2001/18/EC (**Section 5** of this notification).

Expression of the PAT protein confers tolerance to glufosinate-ammonium herbicide that provides the grower with a wider choice for weed control measures. Furthermore, decisions on whether to use herbicides and at what rates do not need to be taken before the emergence of the crop. This should result in effective application of the necessary amounts of glufosinate-ammonium at the most appropriate time. Thus, growers will benefit from a more efficient application of the herbicide while minimising any potential impacts on the environment at the same time.

2. Step 2: Evaluation of the potential consequences of each adverse effect, if it occurs

A comparison of the characteristics of 1507 maize with those of commercially available (non-GM) maize under corresponding conditions of use has assisted in identifying any particular potential adverse effects to human and animal health or the environment arising from the genetic modification (Point **C.2.1.1.** and Point **C.2.1.2.**). Accordingly, the following conclusions have been obtained at **Step 1** of the environmental risk assessment for the placing on the market of 1507 maize:

- There are no identified adverse effects to human and animal health or the environment arising from the genetic modification in 1507 maize;

- There is a limited potential for the development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population regarding cultivation.

The potential consequences from development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population would be limited to causing a reduction in the control of target pests provided by expression of CRY1F protein in 1507 maize.

This would mean reducing the value represented by expression of CRY1F in 1507 maize as a highly effective and environmentally beneficial tool to control certain lepidopteran insect pests, such as the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp.

The CRY1F protein is one of the active ingredients in pesticides based on the *B. thuringiensis* sbsp. *aizawai*, which are used to control wax moth larvae and the diamondback moth caterpillar (*Plutella xylostella*) (Cornell University, 1996). However, these insect pests do not feed on maize plants (the wax moth is one of the main pests of beekeeping and the diamondback moth is a crucifer pest) and they will not be exposed to CRY1F expressed in 1507 maize. Therefore, there is no potential for these insect pests to develop resistance to CRY1F protein and subsequently there will be no adverse effects on the use of pesticides based on the subspecies *aizawai*.

As a result of the above evaluation, we conclude that the magnitude of the potential consequences from the development of resistance to CRY1F protein as expressed in 1507 maize within the target pest population is **limited** and **finite**.

3. Step 3: Evaluation of the likelihood of the occurrence of each identified potential adverse effect

The potential development of insect resistance to other CRY proteins, such as CRY1A(b), in laboratory conditions has been shown following exposure of multiple generations to sublethal concentrations of the toxin. However, field monitoring of insect pests in areas where there is routine application of *B.t.*-based pesticides for several years has not shown development of resistance to the CRY1A(b) protein within the insect target pest populations. Furthermore, no cases of pest resistance to *B.t.*-maize have been reported despite extensive efforts to find them (Andow *et al.*, 1998; Tabashnik *et al.*, 2000).

However, because a few cases of resistance to *B.t.* plants other than *B.t.*-maize have been observed, and although such resistance has been found to be recessive (Tabashnik *et al.*, 2000), the current scientific understanding is that the likelihood of the development of insect resistance to genetically modified maize expressing any CRY proteins, such as 1507 maize expressing CRY1F protein, is **small** but **finite**.

4. Step 4: Estimation of the risk posed by each identified characteristic of the GMO

An estimation of the risk to human health or the environment posed by any identified characteristic of 1507 maize which has the potential to cause adverse effects is made by combining the likelihood of the adverse effect occurring and the magnitude of the consequences, if it occurs.

As mentioned in **Step 1** of this e.r.a., there are no identified adverse effects to human and animal health or the environment arising from the genetic modification in 1507 maize. Therefore, there is no risk to human and animal health or the environment posed by the genetic modification in 1507 maize.

However, there is a limited potential for development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population. The magnitude of the potential consequences of this effect is considered as **limited** and **finite (Step 2)**, whereas the likelihood of this effect occurring is considered as **small** but **finite (Step 3)**. In conclusion, the potential risk of development of insect pest resistance to CRY1F protein as expressed in 1507 maize during cultivation is estimated as **finite** and **limited**.

In the context of responsible product stewardship, a detailed proposal for an insect resistance management (IRM) plan has been developed (Annex 37). The IRM plan will be applied in conjunction with an appropriate monitoring plan (**Section 5**) in order to minimize any potential risks from the placing on the market of 1507 maize including cultivation (**Step 5**).

Overall uncertainty for each identified risk:

As described in this notification, detailed studies on the safety evaluations of CRY1F and PAT proteins confirm that there is no risk to human and animal health or the environment arising from expression of these proteins in 1507 maize. However and as mentioned above, there is a limited potential for development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population. The overall uncertainty for this identified risk is relatively very low and will be managed by the IRM plan. Any other uncertainty is comparable to the uncertainty related to potential risks that might be associated to cultivation of any conventional maize. This is based on the fact that the conclusions reached on **Steps 1** to **4** of the e.r.a. have been founded on the evidence presented and evaluated throughout the notification and on currently available and published scientific literature, as referenced in the text where appropriate. In particular, studies on 1507 maize to establish the molecular organization, expression levels of the CRY1F and PAT proteins, toxicity and allergenicity characteristics, ecotoxicity studies, agronomic performance and compositional analyses confirm that 1507 maize is comparable to other commercial maize except for the expression of CRY1F and PAT proteins, as intended by the genetic modification.

In addition and as mentioned in **Point C.1.g.** above, information from releases of similar organisms and organisms with similar traits and their interaction with similar environments further supports the evidence for a relatively low overall uncertainty

associated to the risk of development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population. In particular, cultivation of Bt-176 maize varieties, expressing CRY1A(b) protein for resistance against certain lepidopteran insect species, over 20,000-25,000 ha each year since 1998 in Spain shows that no resistance to CRY1A(b) protein has been developed within the target insect pest population. In fact, reduction in the use of insecticides to control ECB has resulted in a perceived positive impact to the environment in those areas with high incidence of ECB attack (Brookes, 2002).

5. Step 5: Application of management strategies for risks from the deliberate release for cultivation and marketing of the GMO

The e.r.a. has not identified any risks to human and animal health or the environment arising from the placing on the market of 1507 maize.

However and as described above (**Step 4**), there is a **finite** and **limited** potential risk from the cultivation of 1507 maize. This risk consists of the potential development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population. In response, an appropriate insect resistance management (IRM) plan will be applied in order to minimize any potential risks from the placing on the market of 1507 maize including cultivation.

An industry working group, the EU Working Group on Insect Resistance Management, has developed a harmonised IRM plan specific for cultivation of Bt maize such as 1507 maize in the EU which follows the experiences gained in other countries and takes into account the latest scientific reports. The IRM proposal is entitled 'Harmonised insect resistance plan (IRM) for cultivation of Bt maize in the EU' and it is described in detail in Annex 37 to this notification.

The harmonised IRM plan contains guidance on the following key elements:

- How to use the Bt technology: a comprehensive grower education programme will aid the grower in understanding the importance of insect resistance management to preserve the long-term efficacy of the Bt technology and in employing the required resistance management tool of implementing a generous 20 % refuge for Bt maize planting areas larger than 5 hectares.
- Resistance monitoring: baseline susceptibility of European corn borer (*Ostrinia nubilalis*) and Mediterranean corn stalk borer (*Sesamia nonagrioides*) to Cry1F endotoxin of *B. thuringiensis* in the EU will be measured and monitoring techniques are described to detect changes relative to baseline susceptibility which could result in inadequate protection against *O. nubilalis* and *S. nonagrioides* in the field.
- Potential development of resistance: confirmation of pest resistance and remedial action plan.

In brief, the IRM proposal for the cultivation of 1507 maize consists on the application of the following five principles:

1. Deploying products with an effective dose of Bt protein
2. Maintaining adequate refuges
3. Monitoring product performance
4. Educating seed distributors and farmers
5. Continuing to conduct research

In summary, the notifiers propose to do the following in order to implement the IRM plan:

- encourage farmers growing more than 5 hectares of 1507 maize to plant a generous and conservative 20% refuge with non-Bt maize hybrids;
- limit availability of 1507 maize seed to distributors and sales people to a certain percentage (e.g., 80%) of supplied seed;
- continue to work with scientists in academia to define the appropriate size and distribution of refuges in the different maize growing regions of Europe;
- encourage farmers to continually inspect for product performance (surveillance);
- encourage farmers to report instances of apparent product failure through existing networks (i.e., distributors, sales people and agronomists);
- investigate customer reports of product failure to determine if resistance has developed;
- work with the farmer to implement appropriate remedial actions if insect resistance is confirmed;
- support structured monitoring programs developed by the EU Competent Authorities under Directive 2001/18/EC;
- inform farmers about: *i)* the importance of Bt maize products as part of an integrated pest management system; *ii)* the importance of planting structured refuges and recommendations for the appropriate size of local refuges; and, *iii)* the importance of monitoring for product performance;
- continue to conduct and support research on European corn borer and *Sesamia* biology;
- work with local researchers in the public sector to refine refuge recommendations;
- establish a CRY1F discriminating dose assay and supply CRY1F protein for structured monitoring programs; and,
- continue to develop insect-protection gene products with different modes of action that can be pyramided with the *cry*1F gene.

In addition, the IRM plan will be applied in conjunction with the monitoring plan developed in accordance with Annex VII of Directive 2001/18/EC (**Section 5** of this notification).

Appropriate information on any conditions of use of 1507 maize, including implementation of the IRM and monitoring plan for the cultivation of 1507 maize, will

be provided to agricultural users of 1507 maize by means of labels and/or additional information in the accompanying document, in accordance with Annex IV of Directive 2001/18/EC (Points **A.8.** and **B.7.** of **Section 3** and **Section 6** of this notification).

6. Step 6: Determination of the overall risk of the GMO

The overall risk to human and animal health or the environment arising from the placing on the market of 1507 maize has been evaluated by taking into account the conclusions obtained from the consecutive steps followed in the e.r.a. together with:

(i) The estimated potential risk posed by the expression of CRY1F protein conferring resistance to certain lepidopteran insect pests;

(ii) The proposed insect resistance management plan in the context of product stewardship;

(iii) The proposed monitoring plan to minimize any potential risks from placing on the market of 1507 maize.

*Conclusions from **Step 1** of the e.r.a.:*

There are no identified adverse effects to human and animal health or the environment arising from the genetic modification in 1507 maize;

There is a limited potential for the development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population regarding cultivation.

*Conclusions from **Step 2** of the e.r.a.:*

The magnitude of the potential consequences of the development of insect pest resistance to CRY1F protein is considered **limited** and **finite**.

*Conclusions from **Step 3** of the e.r.a.:*

The likelihood of occurrence of the development of insect pest resistance to CRY1F protein is considered **small** but **finite**.

*Conclusions from **Step 4** of the e.r.a.:*

The potential risk posed during cultivation by the expression of CRY1F protein in 1507 maize is estimated to be **finite** and **limited** for the potential development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population.

An insect resistance management (IRM) plan is proposed in the context of product stewardship, and a monitoring plan is considered appropriate as part of the risk management strategy in order to minimize any potential risks from the placing on the market of 1507 maize including cultivation.

*Conclusions from **Step 5** of the e.r.a.:*

There is negligible risk based on the application of an insect resistance management plan (Annex 37) and the proposal for monitoring (**Section 5** of this notification) for the placing on the market including cultivation of 1507 maize.

Based on the above conclusions obtained in this environmental risk assessment and the information presented throughout this notification including **Part D.** of this environmental risk assessment, we conclude that there is negligible overall risk to human and animal health or the environment from the placing on the market including cultivation of 1507 maize.

D. CONCLUSIONS ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE PLACING ON THE MARKET OF GMOs

D.1. Likelihood of the GMHP becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats

There is negligible likelihood for 1507 maize to become environmentally persistent or invasive giving rise to any weediness. Firstly, because maize does not possess any traits for weediness and secondly, the genetic modification in 1507 maize does not give rise to traits for weediness.

Characteristics for weediness have been generally described by Baker (1974) as (1) the ability for weed seed to germinate in many different environments; (2) discontinuous germination and great longevity of seed; (3) rapid growth through vegetative phase to flowering; (4) continuous seed production for as long as growing conditions permit; (5) self-compatibility but partially autogamous and apomictic; (6) ability to be cross-pollinated by unspecialized visitors or wind-pollinated; (7) high seed output in favorable environments and some seed production in a wide range of environments; (8) adaptation for short and long-distance dispersal; (9) vegetative production or regeneration from fragments and brittleness (hard to remove from the ground); and (10) ability to compete interspecifically by special means.

Maize does not exhibit the above weedy characteristics and is therefore non-invasive in natural ecosystems (Canadian Food Inspection Agency, 1994). Some *Zea* species are successful as wild plants in Central America, but they have no pronounced weedy tendencies. Maize hybrids have been domesticated to the extent that the seeds cannot be separated from the cob and disseminated without human intervention. Maize plants are annuals that generally will not survive in Europe from one growing season to the next because of the poor dormancy and sensitivity to low temperature. Despite its non-dormant nature, maize seed can occasionally persist from one growing season to the next under favorable climatic conditions (see Point **B.3.b.** of **Section 2** of this notification).

In case of unintended release of 1507 maize, current agronomic measures taken to control other commercially available maize can be applied, such as cultivation, selective use of herbicides (with the exception of glufosinate-ammonium herbicide), and crop rotation.

D.2. Any selective advantage or disadvantage conferred to the GMHP

As intended by the genetic modification, specific advantages in agricultural environment have been conferred to 1507 maize: resistance to certain lepidopteran insect pests, such as the European corn borer, and tolerance to glufosinate-ammonium herbicide.

However, maize is highly domesticated, to the extent that it cannot become established as a feral species outside the agricultural environment, and the specific advantages introduced by the genetic modification in 1507 maize do not confer any selective advantage to the plants in the natural environment, *i.e.* outside the agricultural environment. Insect attack is one of the multiple biotic and abiotic factors that prevent growth of maize outside heavily managed agricultural environments, and therefore expression of CRY1F protein conferring resistance to certain lepidopteran insect pests cannot be considered a selective advantage outside the agricultural environment.

Furthermore, application of broad spectrum herbicides, such as glufosinate-ammonium, does not commonly occur outside the agricultural environment, and therefore expression of PAT protein in 1507 maize does not confer a selective advantage outside the agricultural environment.

D.3. Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species

There are no sexually compatible wild or weedy relatives of *Zea mays* known to exist in the EU, which eliminates any potential for gene transfer to such species.

Potential for gene transfer is therefore limited to other maize grown in culture. As discussed in Point **D.2.**, above, the genetic modification in 1507 maize does not introduce any selective advantages to maize plants outside the heavily managed agricultural environments.

D.4. Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP and target organisms, such as predators, parasitoids, and pathogens (if applicable)

The genetic modification in 1507 maize provides growers with a highly effective and environmentally beneficial tool to control certain lepidopteran insect pests, such as the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp.

However, this benefit would be reduced if the target insect pests develop resistance to CRY1F protein as expressed in 1507 maize during cultivation. In the light of current thinking and existing experience with Bt maize products, a detailed proposal for insect resistance management (IRM) has been developed in the context of product stewardship and is attached to this notification (Annex 37). The IRM will be applied in conjunction with the monitoring plan for the placing on the market of 1507 maize, developed in accordance with Annex VII of Directive 2001/18/EC (**Section 5** of this notification).

No other potential immediate and/or delayed environmental impact resulting from direct and indirect interactions of 1507 maize and target organisms in the receiving environment are expected to arise from the placing on the market of 1507 maize.

D.5. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens

The placing on the market of 1507 maize will result in negligible immediate and/or delayed environmental impact resulting from direct and indirect interactions of 1507 maize with non-target organisms, also taking into account organisms which interact with target organisms, including impact on population levels of other organisms.

This conclusion is based on the information presented throughout **Section 2** of this notification, and summarized at **Step 1** of this environmental risk assessment. In particular, it is based on the results obtained from:

i) ecotoxicity studies showing no toxic effects on a range of non-target organisms and beneficial insects, such as green lacewing larvae (*Chrysoperla carnea*), the ladybird beetle (*Hippodamia convergens*), the beneficial parasitic Hymenoptera *Nasonia vitripennis*, honey bee larvae (*Apis mellifera*), earthworms (*Eisenia foetida*), collembola (*Folsomia candida*), the cladoceran aquatic invertebrate *Daphnia magna*, and, the non-target Lepidoptera monarch butterfly (Point **D.10.** of **Section 2** of this notification);

ii) field studies showing no adverse effects of 1507 maize in the population of non-target arthropods (Higgins, 1999; Vernier *et al.*, 2001a, Lefko, 2002; Annexes 30, 36 and 33, respectively);

iii) a detailed non-target exposure and risk assessment for the placing on the market of 1507 maize elaborated by Wolt and Conlan (2001) (Annex 35)

This evidence, together with the specificity of the biological and biochemical activities of the CRY1F and PAT proteins expressed in 1507 maize, confirm the absence of any adverse effects of 1507 maize on the dynamics of populations of non-target organisms in the receiving environment and the genetic diversity of each of these populations.

D.6. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into contact with or in the vicinity of the GMHP release(s)

The genetic modification in 1507 maize does not introduce any new compounds known to cause, or expected to cause, any potential immediate and/or delayed effects on human health resulting from possible direct and indirect interactions of the 1507 maize and persons working with, coming into contact with or in the vicinity of the release of 1507 maize.

This conclusion is based on the evidence presented in Point **D.7.** of **Section 2** of this notification and summarized at **Step 1** of this environmental risk assessment.

D.7. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any products derived from it, if it is intended to be used as animal feed

The genetic modification in 1507 maize does not introduce any new compounds known to cause, or expected to cause, any possible immediate and/or delayed effects on animal health and consumption of 1507 maize and any animal feed products derived from it will result in no adverse consequences for the feed/food chain.

This conclusion is based on a detailed safety evaluation concerning all possible feed applications of 1507 maize and feed products derived from 1507 maize (processed and non-processed) (Annex 1). In summary, feed products from 1507 maize are substantially equivalent to, nutritionally equivalent to, and as safe as, feed products derived from commercially available (non-GM) maize, as confirmed by the compositional analyses comprising protein, fiber, carbohydrates and ash of forage from 1507 maize and protein, fiber, carbohydrates, ash, minerals, fatty acids, amino acids, vitamins, secondary metabolites and anti-nutrients of grain from 1507 maize; nutritional equivalence shown in a poultry feeding study; absence of any toxicologically significant adverse effects in a thirteen-week (90-day) oral toxicity feeding study in rats and, the safety evaluations of the expressed CRY1F and PAT proteins as intended by the genetic modification in 1507 maize.

D.8. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s)

The genetic modification in 1507 maize will not cause any possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of 1507 maize and target and non-target organisms in the vicinity of 1507 maize.

This conclusion is based on the absence of any adverse effects of 1507 maize on non-target and beneficial organisms, and in particular on soil dwelling organisms, such as earthworms (*Eisenia foetida*) and collembola (*Folsomia candida*); on the specificity of toxicity of CRY1F protein to certain target lepidopteran pests; the very limited persistence of the CRY1F protein in the soil environment; the natural ubiquity of the *cry*1F and *pat* genes in the soil environment; and, the specific biochemical activity of PAT protein, as discussed in more detail in **Section 2** of this notification and at **Step 1** of this environmental risk assessment.

D.9. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs

The specific cultivation, management and harvesting techniques used for the 1507 maize are identical to those used for other commercially available (non-GM) maize, with the exception of the application of the IRM plan (Annex 37) and monitoring plan (**Section 5** of this notification) proposed specifically for the cultivation of 1507 maize, thereby limiting the occurrence of any possible immediate and/or delayed, direct and indirect environmental impacts.

As discussed in **Step 1** of this environmental risk assessment, cultivation of 1507 maize expressing CRY1F protein provides farmers with a highly effective and environmentally beneficial tool to control certain lepidopteran insect pests, such as the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp.

Expression of the PAT protein confers tolerance to glufosinate-ammonium herbicide that provides the farmer with a wider choice for weed control measures. Furthermore, decisions on whether to use herbicides and at what rates do not need to be taken before the emergence of the crop. This will provide effective application of the necessary amounts of glufosinate-ammonium at the most appropriate time. Thus, farmers will benefit from a more efficient application of the herbicide while minimising any potential impacts on the environment at the same time.

The only potential risk posed during cultivation of 1507 maize is limited to the potential development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population. However, application of the IRM plan in the context of product stewardship (Annex 37) and the proposed monitoring plan (**Section 5** of this notification) will limit the occurrence of any immediate and/or delayed, direct and indirect, possible impacts from the specific cultivation, management and harvesting techniques used for 1507 maize.

Overall conclusions on the potential environmental impact from the placing on the market of 1507 maize

The e.r.a. has not identified any risks to human and animal health or the environment from the placing on the market of 1507 maize. This is based on the information contained in this notification and the following concluding remarks:

- There is negligible likelihood for 1507 maize to become environmentally persistent or invasive giving rise to any weediness;

- Expression of CRY1F and PAT proteins in 1507 does not confer any selective advantage outside the agricultural environment;

- There are no wild relatives of maize in the EU and the genetic modification in 1507 maize does not introduce any selective advantages to maize plants outside heavily managed agricultural environments;

- The potential reduction of the control of certain lepidopteran insect pests if the target insect pests develop resistance to CRY1F protein as expressed in 1507 maize has been identified as the only potential risk resulting from the interaction of 1507 maize with target organisms;

- The placing on the market of 1507 maize will result in negligible immediate and/or delayed environmental impact resulting from direct and indirect interactions of 1507 maize with non-target organisms;

- The genetic modification in 1507 maize does not introduce any new compounds known to cause, or expected to cause, any potential immediate and/or delayed effects on human health;

- The genetic modification in 1507 maize does not introduce any new compounds known to cause, or expected to cause, any possible immediate and/or delayed effects on animal health and consumption of 1507 maize and any animal feed products derived from it will result in no adverse consequences for the feed/food chain;

- The genetic modification in 1507 maize will not cause any possible immediate and/or delayed effects on biogeochemical processes; and

- The specific cultivation, management and harvesting techniques used for the 1507 maize are identical to those used for other commercially available (non-GM) maize, with the exception of the application of the IRM plan in the context of product stewardship (Annex 37) and monitoring plan (**Section 5** of this notification) proposed specifically for the cultivation of 1507 maize, thereby limiting the occurrence of any possible immediate and/or delayed, direct and indirect impacts to human and animal health or the environment.

E. REVIEW AND ADAPTATION

In accordance with Directive 2001/18/EC the e.r.a. will be reviewed on a regular basis and adapted should relevant new data from ongoing research, other deliberate releases and monitoring become available, that would alter significantly the accuracy of the e.r.a. and the effectiveness of risk management strategies.

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PROPOSAL FOR A MONITORING PLAN FOR THE PLACING ON THE MARKET OF 1507 MAIZE (ANNEX VII, DIRECTIVE 2001/18/EC)

Introduction

The proposal for a monitoring plan for the placing on the market of 1507 maize has been developed according to the principles and objectives outlined in Annex VII of Directive 2001/18/EC and Council Decision 2002/811/EC establishing guidance notes supplementing Annex VII to Directive 2001/18/EC. In particular, the design of the monitoring plan focuses on the cultivation of 1507 maize in the EU based on the conclusions of the environmental risk assessment (see **Section 4**).

The objective of the monitoring plan for the placing on the market of 1507 maize is to confirm the assumptions made and the conclusions obtained in the environmental risk assessment (e.r.a.) (**Section 4** of this notification). In addition, as discussed in detail in **Point 1.1.** below and based on the conclusions of the e.r.a., the monitoring plan is an appropriate tool of the risk management strategy to ensure that the commercial cultivation of 1507 maize poses negligible risk to human and animal health or the environment.

Design of monitoring plan

1. Monitoring strategy

1.1. Risk assessment

An environmental risk assessment (e.r.a.) for the placing on the market of 1507 maize including cultivation has been elaborated in a separate document in accordance with Annex II of Directive 2001/18/EC and it is attached as **Section 4** of this notification.

The product described in this notification is *B.t.* Cry1F maize line 1507, referred to as 1507 maize. It consists of maize product consisting of or derived from seed of 1507 maize genetically modified to express CRY1F protein, conferring resistance to certain lepidopteran insect pests, and PAT protein, conferring tolerance to glufosinate-ammonium herbicide. The maize product also consists of progeny derived from conventional breeding between 1507 maize with any traditionally bred maize.

The e.r.a. is based on specific safety studies and detailed comparisons of the characteristics of 1507 maize with those of commercially available (non-GM) maize under corresponding conditions of use for cultivation. This enables to identify any adverse effects arising from the genetic modification in the assessment of the overall risk to human and animal health or the environment posed by the placing on the market of 1507 maize.

The following conclusions have been obtained from the e.r.a (see **Section 4**):

- There are no identified adverse effects to human and animal health or the environment arising from the genetic modification in 1507 maize, and therefore there is no risk to human and animal health or the environment from placing on the market of 1507 maize.

- There is a limited potential for the development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population regarding cultivation.

- The magnitude of the potential consequences of the development of insect pest resistance to CRY1F protein is considered limited and finite.

- The likelihood of occurrence of the development of insect pest resistance to CRY1F protein is considered small but finite.

- The potential risk from the development of resistance in the target insect pest population to CRY1F protein during cultivation of 1507 maize is estimated as finite and limited.

- An insect resistance management (IRM) strategy is proposed in the context of product stewardship for the cultivation of 1507 maize.

- A monitoring plan is proposed as part of the risk management strategy in order to minimize any potential risks from the placing on the market of 1507 maize including cultivation.

Therefore, a monitoring plan is considered appropriate as part of the risk management strategy and in order to ensure that the commercial cultivation of 1507 maize poses negligible risk to human and animal health or the environment.

1.2. Background information

Characteristics of the GMO, characteristics and scale of the intended use and range of relevant environmental conditions where the GMO is expected to be released

The 1507 maize expresses CRY1F protein conferring resistance to certain lepidopteran insect pests, and PAT protein conferring tolerance to field application of glufosinate-ammonium herbicide. No other traits have been introduced or modified in 1507 maize. The 1507 maize will be used in the EU as any other maize, e.g. by farmers for cultivation, by the animal feed and milling industry, and consumer use by public at large. In 2000, the EU produced 38.4 million tonnes of maize grain. France (43%), followed by Italy (25%), Spain (11%) and Germany (8%) were the main producers. Grain and derived products from 1507 maize are expected to be part of this production.

Maize is widely cultivated in a variety of agricultural environments of the EU, with strong variations at a regional level throughout the Community. The 1507 maize will be cultivated in those environments for which appropriate varieties can be developed.

The 1507 maize would be particularly suitable in environments where there is significant infestation from lepidopteran insect pests, such as Southern and Central Europe.

1.3. Approach

1.3.1. Case-specific monitoring

The e.r.a. concluded that there is a limited potential for development of resistance to CRY1F protein as expressed in 1507 maize within the target pest population. Therefore, a monitoring plan is considered appropriate as part of the risk management strategy to ensure that cultivation of 1507 maize poses negligible risk and in order to maintain the efficacy of the CRY1F protein in 1507 maize, thereby sustaining the environmental benefits of the *Bacillus thuringiensis* (Bt) technology.

The case-specific monitoring plan will form part of the Insect Resistance Management (IRM) proposal entitled 'Harmonised insect resistance plan (IRM) for cultivation of Bt maize in the EU' and described in Annex 37 to this notification. This harmonised IRM plan specific for the EU has been developed by an industry working group, the EU Working Group on Insect Resistance Management, following the experiences gained in other countries and taking into account the latest scientific reports.

The harmonised IRM plan contains guidance on the following key elements:

- How to use the Bt technology: a comprehensive grower education programme will aid the grower in understanding the importance of insect resistance management to preserve the long-term efficacy of the Bt technology and in employing the required resistance management tool of implementing a generous 20 % refuge for Bt maize planting areas larger than 5 hectares.
- Resistance monitoring: baseline susceptibility of European corn borer (*Ostrinia nubilalis*) and Mediterranean corn stalk borer (*Sesamia nonagrioides*) to CRY1F endotoxin of *B. thuringiensis* in the EU will be measured and monitoring techniques are described to detect changes relative to baseline susceptibility which could result in inadequate protection against *O. nubilalis* and *S. nonagrioides* in the field.
- Potential development of resistance: confirmation of pest resistance and remedial action plan.

1.3.2. General surveillance

The cultivation of 1507 maize will result in negligible immediate and/or delayed environmental impact. Furthermore, no significant direct and/or indirect interactions of 1507 maize with non-target organisms are expected, taking into account organisms which interact with target organisms and including impact on population levels of other organisms. This conclusion in the e.r.a. is based on information presented throughout the notification, and in particular the results of the ecotoxicity studies which showed no toxic effects on a range of non-target organisms and beneficial insects. Field studies have also showed no adverse effects of 1507 maize on the population of non-target arthropods. These findings are further elaborated in a detailed non-target exposure and risk assessment (see **Section 4**).

In addition, following the approval for marketing of 1507 maize and in the framework of general surveillance, the notifiers together with relevant experts will reconsider the issues already discussed in the risk assessment with a view to proposing a further study on any unanticipated potential effects of 1507 maize on non-target organisms. The proposed study will take into consideration the latest scientific findings and develop protocols to characterise any unanticipated adverse effects on a series of representative model organisms chosen on the basis of their abundance and/or biological significance in the agricultural environment. As an example, *Orius* spp. could serve as a model species for predators, while other species could serve as appropriate models for other functional groups such as parasitoids and decomposers.

The notifiers will also encourage growers to report any observed adverse effects on non-target arthropod populations or any other potential adverse effects that could derive from changes in the agronomic practices. In addition, national routine surveillance programmes in existence, for example the monitoring of agricultural cultivars or plant protection products (see Annex 38), can be used in cooperation with relevant national organisations and competent authorities for the general surveillance of the occurrence of unanticipated adverse effects relative to changes in conventional agricultural practices.

In addition, since the majority of maize is used for animal feed purposes, general surveillance might assist in confirming the safety of animal feed products derived from 1507 maize with a view to safeguarding against any unanticipated effects. If this is considered necessary, the notifiers can discuss with the relevant national Competent Authorities and associated bodies the co-ordination of a surveillance network, where appropriate, within the framework of their routine surveillance of animal feed products derived from commercial maize.

Facilitation of the observation of the release in the receiving environment and of the interpretation of these observations with respect to human health or the environment

Measures to facilitate case-specific monitoring

In order to monitor for the potential development of resistance to CRY1F protein as expressed in 1507 maize within the target pest population and relating to the insect resistance management proposal, the following measures will facilitate observations and their interpretation during cultivation of 1507 maize (see also Annex 37):

(i) Field collections of target pests to determine baseline susceptibility and contribute to monitor for its variation;

(ii) Monitoring of target pest to detect variation in baseline susceptibility;

(iii) Sampling of plant tissue of maize infested with target pest;

(iv) Sampling of the insect population;

(v) Use of discriminating dose assay (as soon as available) to determine resistant pest phenotype.

Measures to facilitate general surveillance and interpretation of observations

In case of general surveillance the following measures will facilitate the observation and confirmation of any occurrences directly related to the cultivation of 1507 maize (see also Annex 38):

(i) Assisting in insect collections for relevant analyses;

(ii) Encouraging growers to report of any observed adverse effects (including on non-target insects or derived from changes in conventional agricultural practices);

(iii) Providing appropriate test material (CRY1F protein) to laboratories designated to assist in regulatory compliance for monitoring;

(iv) Participating in monitoring programmes developed by the EU Competent Authorities or other relevant national authorities appropriate to 1507 maize (see Annex 38).

1.4. Baselines

Appropriate baselines and/or controls relating to the status of the receiving environment will serve as a point of reference against which any effects arising from the placing on the market of 1507 maize can be compared. This will be determined by parallel monitoring of comparable non-GM maize reference areas (see also *Measures to facilitate case-specific monitoring* above). As mentioned in **Point 1.3.2.** above and following the approval for marketing of 1507 maize, the notifiers together with relevant experts will reconsider the issues already discussed in the risk assessment with a view to proposing a further study on any unanticipated potential effects of 1507 maize on non-target organisms. The proposed study will take into consideration the latest scientific findings and develop protocols that include the characterisation of any unanticipated adverse effects by comparison to the appropriate baselines and/or controls.

In addition, the notifiers can discuss with relevant national organisations and competent authorities, where appropriate, the co-ordination of national routine surveillance programmes in existence, for example the monitoring of agricultural cultivars or plant protection products (see Annex 38), to be used for the general surveillance of the occurrence of unanticipated adverse effects relative to changes in conventional agricultural practices.

1.5. Time-period

Maize is not a persistent crop in the EU environment and as discussed in **Point C.1.c.** of the e.r.a. (**Section 3** of the notification), there are no unexpected agronomic differences between 1507 maize and non-GM maize. In particular, 1507 maize has no altered survival, multiplication or dissemination characteristics. As a result, the time-

period for case-specific monitoring and general surveillance should be proportional to the identified limited potential risk and in line with the period of consent for the placing on the market of 1507 maize *i.e.* ten years. Therefore, it is proposed to submit a first report after three years of the initial placing on the market. Depending on the market introduction, a second report could follow after a second cycle of three years and a final report will be made at the end of the consent.

1.6. Assigning responsibilities

The case-specific monitoring plan for the cultivation of 1507 maize as part of the IRM proposal foresees the following responsibilities:

The notifiers will:

(i) Assist in the determination of base line susceptibility and the monitoring for its variation;

(ii) Instruct growers to scout their 1507 maize growing area and to report unexpected levels of damage from target pests such as the European corn borer and/or *Sesamia* spp. to their sales representative;

(iii) Investigate and identify cause of the damage;

(iv) Initiate and support the development of a discriminating dose assay, which will be used to determine if the pest population in question exhibits a resistant phenotype;

(v) Report all instances of resistance to the competent authority;

(vi) Recommend remedial action to the growers;

(vii) Continue to conduct and support research on target pest biology;

(viii) Work with local researchers in the public sector to refine insect resistance management recommendations;

(ix) Supply CRY1F protein for structured monitoring programmes;

(x) Supply accompanying documentation to growers.

It will be the responsibility of the growers to:

(i) Implement the recommendations contained in the IRM proposal and to incorporate them into their integrated pest management programme;

(ii) Report to the notifiers on any observed adverse effects to the environment;

(ii) Report to the notifiers any cases of product failure;

(iii) Apply any remedial actions recommended by the notifiers and the competent authority, if necessary;

(iv) Forward any required accompanying documentation to purchasers of 1507 maize products.

It will be the responsibility of other users to:

(i) Report to the notifiers on any observed adverse effects to human and animal health or the environment;

(ii) Apply any remedial actions recommended by the notifiers and the competent authority, if necessary.;

(iii) Forward any required accompanying documentation to purchasers of 1507 maize products.

In case of general surveillance, the notifiers will support structured monitoring programmes developed by the competent authorities appropriate to the 1507 maize product (see Annex 38). This could include assisting in non-target insect collections and providing appropriate test material in addition to closely working with the growers and encouraging them to report on any observed adverse effects that may arise from the cultivation of 1507 maize or from changes in conventional agricultural practices. In addition, as mentioned in **Point 1.3.2.** above and following the approval for marketing of 1507 maize, the notifiers together with relevant experts will reconsider the issues already discussed in the risk assessment with a view to proposing a further study on any unanticipated potential effects of 1507 maize on non-target organisms.

1.7. Existing systems

Mechanisms for identifying and confirming any observed adverse effects on human health and the environment

The following mechanisms will be put in place ensuring efficient and workable IRM and monitoring in order to enhance the durability of the insect protected 1507 maize product in the context of responsible product stewardship, and to identify and confirm any observed adverse effects on human health and the environment. Information sharing, education and reporting are being addressed:

(i) Inform growers about the importance of Bt maize products as part of an integrated pest management system;

(ii) Inform growers about the importance of insect resistance management (IRM) and give recommendations for the appropriate implementation of the IRM strategy;

(iii) Inform growers about the importance of monitoring for product performance and encourage them to continually inspect, and carry out general surveillance for potential unanticipated effects on non-target organisms and effects due to changes in conventional agricultural practices;

(iv) Encourage growers to report instances of apparent product failure through existing networks, including results of their general surveillance for potential unanticipated effects on non-target organisms and effects due to changes in conventional agricultural practices;

(v) Investigate grower reports of product failure to determine if resistance has developed using discriminating dose assay when available, including reports of adverse effects following general surveillance on non-target organisms and on changes in conventional agricultural practices;

(vi) Report back any confirmed adverse effects arising from the placing on the market of 1507 maize to the competent authority.

In addition, the notifiers can discuss with relevant national organisations and competent authorities, where appropriate, the co-ordination of national routine surveillance programmes in existence, for example the monitoring of agricultural cultivars or plant protection products (see Annex 38), to be used for the general surveillance of the occurrence of unanticipated adverse effects relative to changes in conventional agricultural practices.

If general surveillance of animal feed products is considered necessary, the notifiers can discuss with the relevant national Competent Authorities and associated bodies the co-ordination of existing mechanisms for general surveillance, where appropriate, within the framework of their routine surveillance of animal feed products derived from commercial maize.

2. Monitoring methodology

2.1 Monitoring parameters/elements

The e.r.a. concluded that there is a limited potential for development of resistance to CRY1F protein as expressed in 1507 maize within the target pest population. As a result and as described in detail in the IRM plan (Annex 37), the relevant parameters/elements to be monitored will consist of: the effects on target organisms, including development of resistance, and product performance.

As discussed above in **Point 1.3.2.**, the notifiers will also encourage growers to report any observed adverse effects on non-target arthropod populations. In addition, the notifiers can discuss with relevant national organisations and competent authorities, where appropriate, the co-ordination of national routine surveillance programmes in existence, for example the monitoring of agricultural cultivars or plant protection products (see Annex 38), to be used for the general surveillance of the occurrence of unanticipated adverse effects relative to changes in conventional agricultural practices.

If general surveillance of animal feed products is considered necessary, the notifiers can discuss with the relevant national Competent Authorities and associated bodies the co-ordination of a surveillance network, where appropriate, within the framework of their routine surveillance of animal feed products derived from commercial maize.

2.2. Areas/samples

As described in detail in **Point 5.2.2.** of the IRM plan (Annex **37**), the relevant areas/samples to be monitored include a representative selection of those where there are heavy ECB (*Ostrinia nubilalis*) and/or PSB (*Sesamia nonagrioides*) infestations and where adoption of this highly effective new Bt technology will be rapid and the planting of insect-protected maize hybrids will be widespread. Both ECB and PSB are important insect pests in most of the Spanish maize growing regions and infestations occur mainly in the Valle del Ebro (Huesca, Zaragoza and Lérida) and in areas of Extremadura and Castilla-La Mancha. In the rest of the EU, ECB is also present at high levels in Northern Italy, Western France and Germany, while PSB infestations generally occur under Mediterranean conditions, *i.e.* below the 45°N parallel.

2.3. Inspections

The inspections to ensure early detection of resistance is a critical part of risk management. Therefore and despite efforts to monitor pest populations for resistance development, it is possible that a grower will be the first person to detect a potential resistant population. To account for this possibility, growers will be instructed to scout their 1507 maize growing area and to report unexpected levels of damage from ECB or PSB to their sales representative and the notifiers will investigate cases of apparent product failure and determine if they are actually due to resistance.

2.4. Sampling and analysis

The sampling and analysis will be carried out by means of a structured monitoring as described in **Point 5.2.2.** of the IRM plan (see the harmonised IRM plan in Annex 37), and will include standard protocols, wherever possible. In particular, the notifiers have supported research work in the U.S. to develop a discriminating dose assay. When available, this assay will be provided to appropriate authorities in the EU. In addition, the notifiers will also support monitoring programs developed by the EU Competent Authorities under Directive 2001/18/EC by assisting in insect collections and providing appropriate test material, where necessary.

2.5. Collection and collation of data

The notifiers will encourage farmers to continually inspect 1507 maize for product performance and to report instances of apparent product failure through existing networks (i.e., distributors, sales people and agronomists). The notifiers will investigate customer reports of product failure to determine if resistance has developed, and will work with the farmer to implement appropriate counter measures if resistance is confirmed. In addition, the notifiers will support structured monitoring programs developed by the EU Competent Authorities under Directive 2001/18/EC.

3. Analysis, reporting, review

3.1. Evaluation

Evaluation of the data with regard to case-specific monitoring and general surveillance will include appropriate statistical analysis, where relevant, and comparisons to baselines and/or controls relating to the status of the receiving environment, in accordance with current scientific knowledge. This will allow for the correct interpretation of the results obtained in order to confirm whether observed environmentally significant changes are in fact due to the 1507 maize or its use, or whether they result from other different environmental factors.

3.2. Reporting

The notifiers will ensure that monitoring and reporting are carried out according to the conditions specified in the consent to the placing on the market of 1507 maize. The reports of this monitoring will be submitted to the Commission and the competent authorities of the Member States. In addition, this information will be made publicly available in line with the requirements of Article 20(4) of the Directive 2001/18/EC.

Frequency

Market introduction of a specific GM crop is a gradual process and the placing on the market might be very small during the initial time period of the consent. Therefore and as discussed in **Point 1.5.** above, it is proposed to submit a first report after 3 years of the initial placing on the market. Depending on the market introduction, a second report could follow after a second cycle of 3 years and a final report will be made at the end of the consent.

Should new information concerning risk become available from users or other sources, the notifiers will immediately take the measures necessary to protect human health and the environment, and inform the competent authority thereof, in accordance with Article 20(2) of Directive 2001/18/EC.

3.3. Review and adaptation

The monitoring plan proposed for the placing on the market of 1507 maize will be reviewed and adapted regularly, on the basis of the information obtained through the monitoring or from other sources and in accordance with the conditions specified in the consent, and, where appropriate, as required by the competent authority.

SECTION 6

PROPOSAL FOR THE LABELLING OF PRODUCTS CONSISTING OF, OR CONTAINING, GENETICALLY MODIFIED 1507 MAIZE ACCORDING TO ANNEX IV OF DIRECTIVE 2001/18/EC

Introduction

The product described in this notification is *B.t.* Cry1F maize line 1507, referred to as 1507 maize, and progeny derived from conventional breeding between 1507 maize with any traditionally bred maize. The product consists of seeds (inbreds and hybrids) of 1507 maize genetically modified to express CRY1F protein, conferring resistance to certain lepidopteran insect pests, and PAT protein, conferring tolerance to glufosinate-ammonium herbicide. In addition, the product consists of material considered as GMO, such as grain produced from 1507 maize seed.

Product information to indicate that genetic modification has been used in the development of 1507 maize will be provided on a label or in an accompanying document. This will enable growers of 1507 maize seed and other users of 1507 maize product in the food and feed chain to label in accordance with the EU legislation.

As specified on Point **A.8** of Annex IV of Directive 2001/18/EC, the information provided on a label or in an accompanying document for the purpose of satisfying the labelling requirements regarding placing on the market of 1507 maize will include the following:

i) Commercial name of the product and the statement that "this product contains genetically modified organisms";

ii) Name of the GMO or the unique identifier code;

iii) Information referred to in Point **A.2.** of Annex IV of Directive 2001/18/EC (name and full address of the notifier established in the Community who is responsible for the placing on the market);

iv) How to access the information in the publicly accessible part of the register.

These specifications will be included in the label or in the accompanying document with regard to 1507 maize products: further details are described below.

However, for products where adventitious or technically unavoidable traces of 1507 maize cannot be excluded but are found to be no higher than a minimum threshold (yet to be established), products will not require labelling according to this proposal.

Proposal for the labelling of 1507 maize seed product

The following information, and in accordance with the requirements of the Marketing of Seeds Directive 66/402/EEC as amended by Directive 98/95/EC, is proposed to be included on the label of 1507 maize seed products placed on the EU market:

- ***Commercial name of the product.*** Commercial names will be assigned to 1507 maize seed or subsequent varieties at the time of market introduction.

- ***This product contains genetically modified organisms (genetically modified variety)***

- ***1507 maize or the unique identifier code (DAS-Ø15Ø7-1)***

- ***Reference to the accompanying document and the public register.*** See below.

Proposal for the labelling of 1507 maize imports

The following information is proposed to be provided to EU grain importers in order to label 1507 maize products placed on the EU market:

- ***Accompanying document.*** See below.

- ***Detection method.*** To identify 1507 maize in imported products and assist with the labelling provisions for placing 1507 maize products on the EU market.

Proposal for the accompanying document

The following information is proposed to be included on the accompanying document for 1507 maize products.

1. Name of the product:

***Commercial name of the* product.** Commercial names will be assigned to 1507 maize seed or subsequent varieties at the time of market introduction. In accordance with the OECD guidance for the designation of a unique identifier for transgenic plants (ENV/JM/MONO(2002)7), the unique identification code assigned to 1507 maize is DAS-Ø15Ø7-1.

2. Name of the manufacturer or distributor:

Pioneer Hi-Bred and Mycogen Seeds are developers of the technology and producers of 1507 maize seed.

Pioneer Hi-Bred International, Inc.
400 Locust Street, Suite 800
Des Moines, IA 50309
U.S.A.

Mycogen Seeds
c/o DowAgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268-1054
U.S.A.

3. Address of the manufacturer or distributor in the EU:

Pioneer Hi-Bred International, Inc. as represented by Pioneer Overseas Corporation:

Pioneer Overseas Corporation
Avenue Tedesco 7
B-1160 Brussels
Belgium

Mycogen Seeds, as represented by Dow AgroSciences Europe:

Dow AgroSciences
2nd floor, 3 Milton Park
Oxon OX14 4RN

United Kingdom

4. Conditions of use of the product:

This product contains genetically modified organisms

The product consists of, or contains, 1507 maize genetically modified to express CRY1F protein, conferring resistance to certain lepidopteran insect pests, and PAT protein, conferring tolerance to glufosinate-ammonium herbicide.

The 1507 maize has been approved for placing on the market including cultivation in the EU under specific conditions of use:

i) ***Implementation of an insect resistance management (IRM) strategy during cultivation of 1507 maize seed;***

ii) ***Specific instructions for carrying out appropriate monitoring and reporting to the notifier and, if required, the competent authority;***

iii) ***Labelling of 1507 maize products in accordance with EU legislation;***

iv) ***Reference to the public register.***

IRM: Implementation of the IRM strategy in the context of product stewardship is required to ensure the durability of the product during cultivation of 1507 maize. A proposal for an IRM plan is attached as a separate document (see Section 4).

Monitoring: Specific instructions for carrying out appropriate monitoring and reporting to the notifier and, if required, to the competent authority. This has been described in detail in the monitoring plan proposed for the cultivation of 1507 maize which is attached as a separate document (see Section 5).

Labelling: Compliance with the labelling requirements in accordance with EU legislation and transmission of these requirements to other users of 1507 maize products.

Public register: Details to be given by the Spanish Competent Authority on how to access the information in the publicly accessible part of the register.

No other restrictions or conditions of use apply to the placing on the market of 1507 maize and therefore products from 1507 maize can be used in a manner consistent with current uses of maize grain and maize products.

Approval for placing on the market of 1507 maize includes cultivation, import for storage and processing, food use, animal feed use and industrial use. The approval for food use of 1507 maize is being considered separately in accordance with Regulation (EC) No 258/97.

Approval for placing on the market of 1507 maize is not restricted to any specific geographical areas within the Community.

Approval for placing on the market of 1507 maize is valid until ...Date for the period of consent to be given by the Spanish Competent Authority. The period for the first consent is requested for a maximum period and should end ten years after inclusion date of the first variety of 1507 maize on an official national catalogue of plant varieties in accordance with Council Directives 70/457/EEC and 70/458/EEC.

Further information is available from the public register at ...Details to be given by the Spanish Competent Authority.

5. *Measures to take in case of unintended release or misuse:*

In case of unintended release of 1507 maize, current agronomic measures taken to control unintended release or misuse of other commercially available maize can be applied, such as cultivation, selective use of herbicides (with the exception of glufosinate-ammonium herbicide), and crop rotation.

6. *Specific instructions for storage and handling:*

No specific instructions for storage and handling of 1507 maize are necessary for the placing on the market of 1507 maize, and therefore grain and grain products of 1507 maize may be stored and handled in the same way as products from other commercial maize varieties.

SECTION 7

SUMMARY NOTIFICATION INFORMATION FORMAT FOR PRODUCTS CONTAINING GENETICALLY MODIFIED HIGHER PLANTS (GMHPs) IN ACCORDANCE WITH DIRECTIVE 2001/18/EC

A. GENERAL INFORMATION

1. Details of notification

(a) Member State of notification:

Spain

(b) Notification number:

C/ES/01/01

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

The product is *B.t.* Cry1F maize line 1507, referred to as 1507 maize, (no commercial names assigned yet). The product consists of maize products derived from seed of genetically modified 1507 maize expressing CRY1F and PAT proteins. The maize product also consists of progeny derived from conventional breeding between 1507 maize with any traditionally bred maize.

(d) Date of acknowledgment of notification:

11th July 2001

2. Notifier

(a) Name of notifier

Pioneer Hi-Bred International, Inc. as represented by Pioneer Overseas Corporation.

Mycogen Seeds, c/o Dow AgroSciences LLC.

This is a joint notification submitted by Pioneer Hi-Bred and Mycogen Seeds. PioneerHi-Bred is taking the lead for this submission.

(b) **Address of notifier**

Pioneer Overseas Corporation
Avenue Tedesco, 7
B-1160 Brussels
Belgium

Pioneer Hi-Bred International, Inc.
400 Locust Street, Suite 800
Des Moines, IA 50309
U.S.A.

Dow AgroSciences Europe
European Development Centre
3 Milton Park, Abingdon
Oxon OX14 4RN
United Kingdom

Mycogen Seeds
c/o DowAgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268-1054
U.S.A.

(c) **Is the notifier**

domestic manufacturer

Pioneer Hi-Bred and Mycogen Seeds are developers of the technology and producers of 1507 maize seed (inbreds and hybrids).

importer

Yes, as importer of seed

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

Same as notifier

3. **General description of the product**

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

The recipient plant is maize (*Zea mays* L.), which is extensively cultivated and has a long history of safe use. The 1507 maize has been genetically modified to express CRY1F protein, conferring resistance to certain lepidopteran insect pests, such as the European corn borer and *Sesamia* spp., and PAT protein, conferring tolerance to glufosinate-ammonium herbicide.

(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**

No, there is no specific form in which 1507 maize must not be placed on the market as a proposed condition of the authorisation applied for.

(c) **Intended use of the product and types of users**

Use of 1507 maize will be consistent with current uses of commercial maize products and in accordance with the monitoring plan (**Section 5** of notification **C/ES/01/01**) and the insect resistance management strategy (**Section 4** of notification **C/ES/01/01**) proposed for the cultivation of 1507 maize. Use of 1507 maize will include cultivation and import of grain and grain products for storage and processing into food, animal feed and industrial uses. Approval for food use of 1507 maize is being considered separately in accordance with Regulation (EC) No. 258/97.

There are multiple categories of users of 1507 maize, e.g. animal feed and milling industry, agriculture, skilled trades and consumer use by public at large. Maize, together with rice and wheat, is one of the most important cereal crops in the world with total production of 596.4 million tonnes in 2000 (FAOSTAT Database, 2000). The FAO estimation for the EU maize production in 2000 was 38.4 million tonnes. Majority of grain and forage derived from maize is used for animal feeds, and about 8% of the grain is processed for human food products mainly by wet-milling or dry-milling. Maize grain is also processed into industrial products (11%), such as ethyl alcohol by fermentation and highly refined starch by wet-milling to produce starch and sweetener products. In addition to milling, the maize germ can be processed to obtain maize oil.

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

Use of 1507 maize will be consistent with current uses of maize products and in accordance with the monitoring plan (**Section 5** of notification **C/ES/01/01**) and the insect resistance management strategy (**Section 4** of notification **C/ES/01/01**) developed for the cultivation of 1507 maize. Labelling of 1507 products will be carried out in accordance with EU legislation. See **Point A.3.h)** below for labelling of 1507 maize.

(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**

The 1507 maize would be particularly suitable in environments where there is infestation from lepidopteran insect pests, such as Southern and Central Europe, provided specific varietal germplasm with appropriate maturity is available.

(g) **Any proposed packaging requirements**

The packaging, handling, and storage systems that are currently used for maize will apply. The 1507 maize products will be packaged in the same manner as

products from other commercial maize. See **Point A.3.h)** below for labelling of 1507 maize.

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

Product information to indicate that genetic modification has been used in the development of 1507 maize will be provided on a label or in an accompanying document. A proposal for the labelling of products consisting of, or containing, genetically modified 1507 maize has been prepared in accordance with Annex IV of Directive 2001/18/EC (**Section 6** of notification **C/ES/01/01**). This will enable 1507 maize products to be labelled appropriately in accordance with Directive 2001/18/EC.

(i) Estimated potential demand

(i) in the Community

Import of maize grain into the EU for 2000 was about 10.3 million tonnes and the EU maize production including cultivation was 38.4 million tonnes (FAOSTAT Database, 2002). The potential demand for 1507 maize in the Community is expected to be part of these imports and production.

(ii) in export markets for EC supplies

Maize grain is traded as a commodity and no specific demand for 1507 maize should be expected from export markets for EC supplies.

(j) Unique identification code of the GMO

In accordance with the OECD guidance for the designation of a unique identifier for transgenic plants (ENV/JM/MONO(2002)7), the unique identification code assigned to 1507 maize is DAS-Ø15Ø7-1.

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, 1507 maize has been notified in Italy, France and Spain for field trials under Part B of Directive 90/220/EEC.

Year	Member State	Notification No
1998	Italy	B/IT/98/19
1999	Italy	B/IT/98/19
1999	France	B/FR/99.03.09
2000	Italy	B/IT/98/19
2000	France	B/FR/99.03.09
2000	France	B/FR/00.03.04
2002	Spain	B/ES/02/11

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

Not applicable.

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

Yes, a separate notification (Ref. **C/NL/00/10**) to import 1507 maize has been submitted to The Netherlands in accordance with Directives 90/220/EEC and 2001/18/EC. In addition a separate application has been submitted to The Netherlands for food use of 1507 maize in accordance with Regulation (EC) No 258/97.

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

Yes, an application for registration of 1507 maize was submitted to the US Environment Protection Agency (EPA). Also, an application for non-regulated status of 1507 maize to the US Department of Agriculture (USDA) was submitted in May 2000, and a notification concerning foods derived from 1507 maize to the US Food and Drug Administration (FDA) was submitted in July 2000. The corresponding permits were granted as follows: by US EPA and FDA on 18th May 2001 and by USDA on 14th June 2001.

In addition, applications have been submitted to Argentina, Australia/New Zealand, Canada, China, Japan, Korea, Mexico, South Africa, Switzerland and Taiwan. The necessary approvals for import, animal feed use and food safety of 1507 maize in Japan were obtained on 15th June, 28th May and 8th July of 2002, respectively. In Canada permits were granted by Health Canada for novel food use of 1507 maize on 10th October 2002 and by the Canadian Food Inspection Agency for animal feed use and environmental release on 11th October 2002. Approval for import of 1507 maize for animal feed and food use in South Africa was obtained on 12th December 2002.

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

No.

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

Based on the conclusions from the environmental risk assessment (e.r.a.) for the placing on the market of 1507 maize in accordance to Annex II of Directive 2001/18/EC (**Section 4** of notification **C/ES/01/01**) no specific measures need to be taken in case of unintended release or misuse or for disposal and treatment.

In case of unintended release of 1507 maize, current agronomic measures taken to control unintended release or misuse of non-GM maize can be applied, such as cultivation, selective use of herbicides (with the exception of glufosinate-ammonium herbicide), and crop rotation.

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:	Gramineae
(b) Genus:	*Zea*
(c) Species:	*Z. mays* L.
(d) Subspecies:	None
(e) Cultivar/breeding line:	Line Hi-II
(f) Common name:	Maize; corn

9. a) Information concerning reproduction

(i) Mode(s) of reproduction

As a wind-pollinated, monoecious grass species, self-pollination and fertilisation and, cross-pollination and fertilisation, are usually possible and frequencies of each are normally determined by proximity and other physical influences on pollen dispersal.

(ii) Specific factors affecting reproduction, if any

Tasselling, silking, and pollination are the most critical stages of maize development, and grain yield is greatly impacted by moisture and fertility stress. Dispersal of maize pollen tends to be limited, as it is influenced by the large size and rapid settling rate of the pollen.

(iii) Generation time

Maize is an annual crop with a cultural cycle ranging from as short as 10 weeks to as long as 48 weeks covering the period of seedling emergence to maturity. This variance in maturity allows maize to be grown over a range of climatic conditions.

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

Maize will intra-pollinate and will not transfer genetic material to other plant species in the EU. The extent of pollination will depend upon prevailing wind patterns, humidity and temperature. It is generally considered that teosinte (*Zea mays* ssp. *mexicana*) is an ancestor of maize. Teosinte is an ancient wild grass found in Mexico and Guatemala and is not present in the EU.

10. Survivability

(a) Ability to form structures for survival or dormancy

During the domestication of maize, many agronomically significant attributes for cultivation have been gained whilst losing its ability to survive in the wild. Maize is a non-dormant annual crop and seeds are the only survival structures. Natural regeneration of maize from vegetative tissue is not known to occur.

(b) Specific factors affecting survivability, if any

Survival of maize seed is dependent upon temperature, moisture of seed, genotype, husk protection and stage of development. Maize seed can only survive under favourable climatic conditions. Freezing temperatures have an adverse effect on germination of maize seed and it has been identified as a major risk in limiting production of maize seed.

11. Dissemination

(a) Ways and extent of dissemination

Maize has a polystichous female inflorescence (ear) on a stiff central spike (cob) enclosed in husks (modified leaves). As a result, seed dispersal of individual kernels does not occur naturally.

(b) Specific factors affecting dissemination, if any

Mechanical harvesting and transport are ways of disseminating grain and insect or wind damage may cause mature ears to fall to the ground and avoid harvest. Regardless of these routes of dissemination, maize cannot survive without human assistance.

12. Geographical distribution of the plant

Maize is grown over a wide range of climatic conditions because of its many divergent types. However, survival and reproduction in maize is limited by cool conditions. The greatest maize production occurs where the warmest month isotherms range between 21 and 27°C and the freeze-free season lasts 120 to 180 days. Maize has been cultivated in Europe starting in Southern Europe since the 16th century.

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 maize has been cultivated in Europe since the 16th century.

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

Maize is known to interact with other organisms in the environment including insects, birds, and mammals. It is susceptible to a range of fungal diseases and insect pests, as well as competition from surrounding weeds. Maize is extensively cultivated and has a history of safe use. Maize or derived products of maize are not considered to have harmful characteristics. Maize has no pathogenic characteristics.

15. Phenotypic and genetic traits

Maize (*Zea mays* L.) is the only species usually included in the genus Zea, of the family Gramineae. It is a highly domesticated agricultural crop with well-characterised phenotypic and genetic traits.

Controlled cross-pollination of inbred lines from chosen genetic pools combines desired genetic traits in a hybrid resulting in improved agronomic performance and yield increase. This inbred-hybrid concept and resulting yield response is the basis of the modern maize seed industry.

INFORMATION RELATING TO THE GENETIC MODIFICATION

16. Description of the methods used for the genetic modification

The particle acceleration method was used to introduce into maize cells a linear DNA fragment containing the *cry*1F and *pat* coding sequences and the necessary regulatory components (insert PHI8999A). Maize event 1507 expressing the CRY1F protein and the PAT protein was produced, referred to as 1507 maize.

17. Nature and source of the vector used

No vector was used for the transformation of 1507 maize. As described in the notification, the intended insert consists of a linear DNA fragment, containing the *cry*1F and *pat* coding sequences together with the necessary regulatory components only, which was introduced by particle acceleration for the transformation of 1507 maize. No additional DNA sequences were used for introduction of the insert into 1507 maize.

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

The intended insert PHI8999A contains the plant optimised coding sequences for the *cry*1F and *pat* genes, together with the necessary regulatory components to drive their expression.

The *cry*1F gene (1818 bp; origin: *Bacillus thuringiensis* subsp. *aizawai*) is under the control of the ubiquitin promoter *ubi*ZM1(2) (1986 bp; origin: *Zea mays*) and the ORF25PolyA terminator (714 bp; origin: *Agrobacterium tumefaciens* pTi15995). The function of the CRY1F protein in 1507 maize is to provide resistance against certain lepidopteran insect pests such as the European corn borer and *Sesamia* spp.

The *pat* gene (552 bp; origin: *Streptomyces viridochromogenes* strain Tü494) is under the control of the CaMV35S promoter and terminator (554 and 204 bp, respectively; origin: cauliflower mosaic virus). The function of the PAT protein in 1507 maize is to tolerate application of glufosinate-ammonium herbicide.

INFORMATION RELATING TO THE GMHP

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

The 1507 maize expresses CRY1F protein conferring resistance to certain lepidopteran insect pests, and PAT protein conferring tolerance to glufosinate-ammonium herbicide.

The *cry*1F gene is expressed constitutively by the *ubi*ZM1(2) promoter. Expression of CRY1F protein provides control against lepidopteran insect pest damage to the above-ground parts of the maize plant including those parts which are beyond the reach of chemical insecticides. Specifically, the CRY1F protein confers season-long resistance against the European corn borer (*Ostrinia nubilalis*) and certain other lepidopteran pests such as the pink borer (*Sesamia* spp.). It is also highly effective against fall armyworm (*Spodoptera frugiperda*), black cutworm (*Agrotis ipsilon*) and southwestern corn borer (*Diatraea grandiosella*).

The *pat* gene is also expressed constitutively by the CaMV35S promoter. Expression of PAT protein confers tolerance to application of glufosinate-ammonium herbicide. Field trials show that 1507 maize will tolerate field application rates of 1600 g a.i./ha of glufosinate-ammonium herbicide without showing any phytotoxicity symptoms. Tolerance to glufosinate-ammonium herbicide provides for improved weed management.

No other new traits have been introduced into 1507 maize and, in particular, no trait for antibiotic resistance is present in 1507 maize. As discussed in detail throughout the notification (**C/ES/01/01**), these characteristics of 1507 maize have been confirmed by molecular characterization, protein expression analysis,

agronomic performance, and comparison of composition data to other conventional non-GM maize.

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 genetic modification in 1507 maize has been characterised in detail by Southern blot and DNA sequence analyses. The analyses have confirmed that the inserted genetic material is integrated into the nuclear genome of the maize plant and consists of an almost full-length copy of the linear fragment used in the transformation (*i.e.*, 6186 bp from the 6235 bp of insert PHI8999A, containing the *cry*1F and *pat* genes together with the regulatory sequences necessary for their expression). In addition, the plant insert contains the following non-functional fragments:

- one fragment (335 bp) of the *cry*1F gene, with no *ubi*ZM1(2) promoter sequence, and one fragment (15 bp) of the *cry*1F gene, both located at the 5' end of the almost full-length insert;
- two fragments (201 bp and 138 bp long, respectively) of the *pat* gene, without regulatory sequences associated, located at the 5' border and, one fragment (188 bp) of the *pat* gene, located at the 3' border;
- one fragment (118 bp) of the polylinker region and *ubi*ZM1(2) promoter sequence located at the 5' border;
- one fragment (550 bp) of the ORF25PolyA terminator sequence in inverted position located immediately at the 3' end of the almost full-length insert.

The 1507 maize does not contain the *nptII* gene nor any other detectable fragments from the portion of plasmid PHP8999 that was not intended for transformation of 1507 maize. Maize genomic DNA flanking regions at both the 5' and 3' borders of the 1507 maize insert have been sequenced and characterised in detail. Analysis by PCR amplification has confirmed the presence of both maize genomic flanking regions in non-GM Hi-II maize used in the transformation of 1507 maize.

(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, chloroplasts, mitochondrion, or maintained in a non-integrated form), and methods for its determination

The inserts are integrated into the maize plant genome as confirmed in the molecular characterisation of 1507 maize by detailed Southern blot analysis and DNA sequencing.

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

As discussed in **Point B.20.(a)** above, the insert integrated in 1507 maize contains one copy of the almost full-length linear fragment used in the transformation, which includes one functional copy of the complete *cry*1F gene and one functional copy of the complete *pat* gene, together with the regulatory sequences necessary for their expression. The insert also contains two non-functional fragments of the *cry*1F gene; three non-functional fragments of the *pat* gene; one non-functional fragment of the polylinker region and *ubi*ZM1(2) promoter; and, one non-functional fragment of the ORF25PolyA terminator sequence.

The inserted genetic material in 1507 maize is stable for at least six generations, and the *cry*1F and *pat* genes are inherited as Mendelian dominant genes. Results from Southern blot and DNA sequencing analyses show that the additional non-functional fragments were present in the BC4 backcross generation, thus supporting the conclusion that they are genetically linked to the almost full-length insert containing the *cry*1F and *pat* genes.

(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**

Leaf, pollen, silk, stalk, whole plant, grain and senescent whole plant tissue samples from 1507 maize and control maize with comparable background genetics were obtained from field studies conducted during the growing seasons of 1998/99 in Chile, 1999 in France and Italy, and 2000 in France, Italy and Bulgaria. Expression levels of CRY1F and PAT proteins in these tissues were measured using specific Enzyme Linked Immunosorbent Assay (ELISA) developed for each protein. Results show that the CRY1F protein is expressed in all tissues and throughout the development of maize, while the PAT protein was measurable at the V9 developmental stage only.

The characteristics of the CRY1F and PAT proteins expressed in 1507 maize were further examined by Western blot analysis. The CRY1F protein was detected as two bands of approximately 65 and 68 kDa, respectively, which result from limited N-terminal processing of maize expressed CRY1F protein by a plant protease with trypsin-like specificity. No other bands indicative of a partial CRY1F protein or a fusion protein of greater molecular weight were observed.

The PAT protein is known to be a homodimer of approximately 43 kDa in its native form, and it is comprised of two components of approximately 22 kDa. The results of the Western blot analysis of 1507 maize confirmed the presence of

the ~22 kDa PAT monomeric form and of the ~43 kDa PAT homodimer in leaf tissue. No other bands indicative of a partial PAT protein or fusion protein of greater molecular weight were observed in 1507 maize.

The genetic modification in 1507 maize results in expression of CRY1F protein conferring resistance to certain lepidopteran insect pests, and PAT protein conferring tolerance to glufosinate-ammonium herbicide. Specifically, the CRY1F protein confers season-long resistance against the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. It is also highly effective against fall armyworm (*Spodoptera frugiperda*), black cutworm (*Agrotis ipsilon*) and southwestern corn borer (*Diatraea grandiosella).*

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

Addressed in **Point B.21.(a)** above.

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

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

No unexpected changes in pollen production, seed production, seed viability or germination compared to non-GM maize have been observed in field trials of 1507 maize.

(b) Dissemination

Maize hybrids have been domesticated to the extent that the seeds cannot be disseminated without human intervention. The 1507 maize plants show no difference in dissemination compared to non-GM maize.

(c) Survivability

Cultivated maize has been domesticated to the extent that it can not survive outside managed agricultural environments. Lack of dormancy prevents maize seed to readily survive from one growing season to the next. The genetic modification in 1507 maize results in expression of CRY1F conferring resistance to certain lepidopteran insect pests and expression of PAT conferring tolerance to the herbicide glufosinate-ammonium. The survival characteristics of 1507 maize in the environment remain comparable to those of non-GM maize.

(d) Other differences

Maize does not exhibit any weedy tendencies and is non-invasive in natural ecosystems. Based on the agronomic data, there is no evidence for altered survival, multiplication, or dissemination of 1507 maize in the environment as compared to non-GM maize. In addition, the inserted traits do not alter the phenotype of maize in a way that would confer a fitness advantage for maize outside managed agricultural environments.

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

The potential for transfer of genetic material from 1507 maize to other organisms will be negligible as there are no sexually compatible wild or weedy relatives of *Zea mays* known to exist in the EU.

Transfer of genetic material from 1507 maize to bacteria is a negligible concern. There is no known mechanism for, or definitive demonstration of, DNA transfer from plants to microbes under natural conditions. Even if horizontal gene transfer were to take place, transfer of the *cry*1F or *pat* gene from 1507 maize does not represent a risk to human or animal health nor is it of consequence as a plant pest risk. The *nptII* gene coding for resistance to the antibiotic kanamycin is not present in 1507 maize.

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

(i) Toxicity

The genetic modification in 1507 maize results in expression of CRY1F and PAT proteins. The CRY1F protein has specific toxicity against certain lepidopteran insect pests (target organisms). An acute toxicity study with CRY1F protein in mice has confirmed the safety of the CRY1F protein to human and animal health. No mortality, toxicity or adverse clinical signs were observed at the highest dose tested of 5050 mg of test material per kg of body weight which was equivalent to 576 mg of pure CRY1F protein per kg of body weight. In addition, there is no evidence for CRY proteins originating from *Bacillus thuringiensis* to have harmful effects on the health of humans and animals.

The safety in terms of toxicity for the PAT protein has already been determined in detail during the assessment of glufosinate-ammonium tolerant maize. The *pat* gene was originally obtained from *Streptomyces viridochromogenes* strain Tü494 which has no known toxic or pathogenic potential. Toxicity studies carried out on rats and mice containing up to 50000 and 5000 mg/kg body weight respectively, have confirmed the absence of any adverse treatment-related clinical signs.

In addition, a poultry feeding study over a period of 42 days has been carried out confirming that there are no statistically significant differences on mortality, body weight gain or feed conversion between chickens fed a diet containing grain from 1507 maize or from non-GM maize.

(ii) Allergenicity

The most important factor to consider in assessing allergenic potential is whether the source of the gene being introduced into plants is known to be allergenic. Neither *Bacillus thuringiensis* (the source of the *cry*1F gene) nor *Streptomyces*

viridochromogenes (the source of the *pat* gene) have a history of causing allergy. Also, both donor organisms are common soil bacteria.

The assessment of the allergenic potential of the CRY1F and PAT proteins has been made following the recommendations and the application of the decision-tree from FAO/WHO. The analyses have consisted of amino acid sequence comparison with known allergens, rapid degradation in simulated gastric fluids, relatively low level of expression, lack of glycosylation and thermolability. The results confirm that CRY1F and PAT proteins do not pose any significant risk of being a potential allergen.

(iii) Ecotoxicity studies

The absence of toxicity of the CRY1F protein to non-target and beneficial organisms has been thoroughly assessed in multiple dietary toxicity studies including green lacewing larvae, ladybird beetle, beneficial parasitic Hymenoptera *Nasonia vitripennis*, Monarch butterfly larvae, honey bees, earthworm, collembola and daphnia. In addition, levels of beneficial arthropods in field plots of 1507 maize were found to be comparable to those observed in non-GM maize.

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

A detailed safety evaluation concerning possible feed applications of 1507 maize and feed products derived from 1507 maize (processed and non-processed) has been carried out (**Annex 1** to **Section 2** of notification **C/ES/01/01**).

The conclusions obtained confirm that feed products from 1507 maize are substantially equivalent to, nutritionally equivalent to, and as safe as, feed products derived from commercially available (non-GM) maize. This is based on compositional analyses comprising protein, fiber, carbohydrates and ash of 1507 maize forage and on compositional analyses comprising protein, fiber, carbohydrates, ash, minerals, fatty acids, amino acids, vitamins, secondary metabolites and anti-nutrients in grain samples from 1507 maize; the nutritional equivalence shown in a poultry feeding study; and, the detailed safety evaluation of the CRY1F and PAT proteins expressed in 1507 maize.

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

The mechanism of interaction between CRY1F protein expressed in 1507 maize and target organisms can be summarized as follows:

Maize expressed CRY1F protein consists of residues 1 to 605 of the native CRY1F sequence from *B. thuringiensis* sbsp. *aizawai*, with a single and conservative amino acid substitution (F to L at position 604). Upon ingestion of 1507 maize tissue by susceptible insects (target pests) the maize expressed CRY1F protein will reach the alkaline conditions of the insect gut where proteolytic processing of

CRY1F protein by trypsin-like proteases may occur before it binds to specific receptors on the apical microvilli of epithelial midgut cells of the insect and the CRY1F protein undergoes a conformational change that allows insertion into the membrane of the cell. Protein oligomerization will then occur with formation of pores in the membrane of the midgut cells of the insect causing osmotic cell lysis leading to insect death.

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

There are no potentially significant changes in the interactions of 1507 maize with non-target organisms resulting from the genetic modification. As discussed in detail in **Point D.10.** of **Section 2** and **Points C.1.c** and **D.5.** of **Section 4** of the notification (**C/ES/01/01**), the specific biological activity of CRY1F and PAT proteins expressed in 1507 maize together with the absence of toxicity of CRY1F protein to non-target and beneficial organisms provides strong evidence for the absence of any significant toxicity to non-target organisms which may arise from exposure to 1507 maize.

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

The 1507 maize can be detected by placing small amounts of the glufosinate-ammonium herbicide on leaves of maize plants. Maize plants with the expression of the PAT protein will be those with leaves that do not show any necrosis at point of herbicide application. Alternatively, maize plants can be sprayed with glufosinate-ammonium herbicide, and those that survive will be expressing the PAT protein.

Plant parts of 1507 maize can also be analysed by ELISA to detect the proteins expressed by the *cry*1F and *pat* genes. Additionally, an insect bioassay with sensitive lepidopteran insect species such as European corn borer (*Ostrinia nubilalis)* can be used to identify maize plants expressing the CRY1F protein.

A PCR detection method to confirm the molecular identity of 1507 maize has been developed. The PCR method can also be used to confirm presence of 1507 maize for the purposes of labelling products containing or derived from 1507 maize. The detection method for 1507 maize has been provided to the Spanish regulatory authority. The PCR detection method and reference material will be available to a central body operating under the auspices of the EU regulatory authorities and the European Commission.

A combination of the techniques described above can be used to identify 1507 maize.

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 GMO(s) (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)

A comparison of the characteristics of 1507 maize with those of commercially available (non-GM) maize under corresponding conditions of use has assisted in identifying any particular potential environmental impact from the placing on the market of 1507 maize. A detailed assessment has been carried out in accordance to Annex II, D2 of Directive 2001/18/EC (**Points D.1.** to **D.9.** of **Section 4** of notification **C/ES/01/01**), which has lead to the following conclusions:

- There is negligible likelihood for 1507 maize to become environmentally persistent or invasive giving rise to any weediness;

- Expression of CRY1F and PAT proteins in 1507 maize does not confer any selective advantage outside the agricultural environment;

- There are no wild relatives of maize in the EU and the genetic modification in 1507 maize does not introduce any selective advantages to maize plants outside managed agricultural environments;

- The potential reduction of the control of certain lepidopteran insect pests if the target insect pests develop resistance to CRY1F protein as expressed in 1507 maize has been identified as the only potential risk resulting from the interaction of 1507 maize with target organisms;

- The placing on the market of 1507 maize will result in negligible immediate and/or delayed environmental impact resulting from direct and indirect interactions of 1507 maize with non-target organisms;

- The genetic modification in 1507 maize does not introduce any new compounds known to cause, or expected to cause, any potential immediate and/or delayed effects on human health;

- The genetic modification in 1507 maize does not introduce any new compounds known to cause, or expected to cause, any possible immediate and/or delayed effects on animal health and consumption of 1507 maize and any animal feed products derived from it will result in no adverse consequences for the feed/food chain;

- The genetic modification in 1507 maize will not cause any possible immediate and/or delayed effects on biogeochemical processes; and,

- The specific cultivation, management and harvesting techniques used for the 1507 maize are identical to those used for other commercially available (non-GM) maize, with the exception of the application of the IRM strategy in the context of

product stewardship and of the monitoring plan proposed specifically for the cultivation of 1507 maize, thereby limiting the occurrence of any possible immediate and/or delayed, direct and indirect impacts to human and animal health or the environment.

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)

The environmental risk assessment (e.r.a.) for the placing on the market of 1507 maize has concluded that there might be a limited potential environmental impact derived from the interaction between 1507 maize and target organisms consisting of the potential development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population. However, and in order to ensure that placing on the market of 1507 maize poses negligible risk to the environment, appropriate monitoring and risk management proposals have been developed for application following approval of 1507 maize.

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

Placing on the market of 1507 maize will result in negligible immediate and/or delayed environmental impact resulting from potential interactions of 1507 maize with non-target organisms. In particular, no significant adverse effects on biodiversity will occur in the area of cultivation. This conclusion is based on the information presented in **Section 2** and **Section 4** of the notification (**C/ES/01/01**), and, in particular, on the results obtained from:

i) ecotoxicity studies showing no toxic effects of CRY1F protein on a range of non-target organisms and beneficial insects, such as green lacewing larvae (*Chrysoperla carnea*), the ladybird beetle (*Hippodamia convergens*), the beneficial parasitic Hymenoptera *Nasonia vitripennis*, honey bee larvae (*Apis mellifera*), earthworms (*Eisenia foetida*), collembola (*Folsomia candida*), the cladoceran aquatic invertebrate *Daphnia magna*, and, the non-target Lepidoptera Monarch butterfly;

ii) field studies showing no significant adverse effects of 1507 maize in the population of non-target arthropods;

iii) a detailed non-target exposure and risk assessment for the placing on the market of 1507 maize.

Such evidence, together with the specificity of the biological and biochemical activities of the CRY1F and PAT proteins expressed in 1507 maize, confirms that 1507 maize will have negligible effects on the dynamics or biodiversity of populations of non-target organisms in the area of cultivation.

b) Effects on biodiversity in other habitats

There will be negligible effects on the dynamics or biodiversity of populations of non-target organisms in other habitats. This is based on the fact that maize has no wild relatives in the EU and the genetic modification in 1507 maize has not altered the lack of potential for dispersal or weediness of maize. In particular, the genetic modification in 1507 maize has not introduced any selective advantages outside managed agricultural habitats. Also, the lack of ability by the genetic modification in 1507 maize to transfer genetic material to other organisms has not been altered, and, the genetic modification in 1507 maize has not introduced any new compounds known to be toxic, allergenic or harmful to human or animal health.

c) Effects on pollinators

As mentioned in **Point B.31.b** above, expression of CRY1F protein in 1507 maize has no toxic effects on non-target organisms and beneficial insects and it is highly specific against certain lepidopteran insect pests that feed on maize plant tissues, such as the European corn borer and *Sesamia* spp. As a result, cultivation of 1507 maize will have negligible effects on pollinators.

d) Effects on endangered species

The geographic distribution, habitat preferences and host plants of endangered insect species are not predominantly associated with agricultural environments such as those used for maize cultivation. The habitat preferences for the non-target endangered species include marshy, arid, semiarid or alpine (> 1000 m elevation) environments. Such habitat preferences and distribution confirm that endangered species will not be significantly affected from cultivation of 1507 maize.

In addition, a recent publication by Hellmich *et al.* (2001) has confirmed that CRY1F protein from 1507 maize is relatively non-toxic to Monarch larvae compared to other CRY proteins and that pollen from 1507 maize will have no acute effects on Monarch butterfly larvae in field settings. These results further confirm the high degree of specificity of the biological activity of CRY1F protein expressed in 1507 maize against certain target lepidopteran insect pests.

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**
B/IT/98/19

(b) **Conclusions of post-release monitoring**
The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(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 observed.

(a) **Notification number**
B/FR/99.03.09

(b) **Conclusions of post-release monitoring**
The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(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 observed.

(a) **Notification number**
B/ES/02/11

(b) **Conclusions of post-release monitoring**
The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(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 observed.

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

(a) **Release country**
Argentina.

(b) **Authority overseeing the release**
Secretary of Agriculture.

(c) **Release site**
Pergamino area, 3 sites; Buenos Aires Province.

(d) **Aim of the release**
Efficacy trials and hybrid registration.

(e) **Duration of the release**
One season.

(f) **Aim of post-release monitoring**
Control of potential volunteers.

(g) **Duration of post-release monitoring**
One season.

(h) **Conclusions of post-release monitoring**
The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(i) **Results of the release in respect to any risk to human health and the environment**
No adverse effects on human health and the environment observed.

(a) **Release country**
Brazil

(b) **Authority overseeing the release**
CTNBio

(c) **Release site**
One site.

(d) **Aim of the release**
Research.

(e) **Duration of the release**
One season.

(f) **Aim of post-release monitoring**
Control of potential volunteers.

(g) **Duration of post-release monitoring**
One season.

(h) **Conclusions of post-release monitoring**
The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(i) **Results of the release in respect to any risk to human health and the environment**
No adverse effects on human health and the environment observed.

(a) **Release country**
Chile.

(b) **Authority overseeing the release**
Ministry of Agriculture.

(c) **Release site**
Four sites.

(d) **Aim of the release**
Research.

(e) **Duration of the release**
One season.

(f) **Aim of post-release monitoring**
Control of potential volunteers.

(g) **Duration of post-release monitoring**
One season.

(h) **Conclusions of post-release monitoring**
The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.

(i) **Results of the release in respect to any risk to human health and the environment**
No adverse effects on human health and the environment observed.

(a) **Release country**
South Africa.
(b) **Authority overseeing the release**
Ministry of Agriculture.
(c) **Release site**
One site.
(d) **Aim of the release**
Research.
(e) **Duration of the release**
One season.
(f) **Aim of post-release monitoring**
Control of potential volunteers.
(g) **Duration of post-release monitoring**
One season.
(h) **Conclusions of post-release monitoring**
The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.
(i) **Results of the release in respect to any risk to human health and the environment**
No adverse effects on human health and the environment observed.

(a) **Release country**
U.S.A.
(b) **Authority overseeing the release**
USDA and EPA.
(c) **Release site**
Multiple sites.
(d) **Aim of the release**
Research.
(e) **Duration of the release**
Five seasons.
(f) **Aim of post-release monitoring**
Control of potential volunteers.
(g) **Duration of post-release monitoring**
One season.
(h) **Conclusions of post-release monitoring**
The 1507 maize plants performed as expected, with no evidence of any unintentional morphological or phenotypical characteristics. In particular, there was no evidence of enhanced weediness of 1507 maize.
(i) **Results of the release in respect to any risk to human health and the environment**
No adverse effects on human health and the environment observed.

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

A monitoring plan has been considered appropriate as part of the risk management strategy in order to minimize any potential risks from the placing on the market of 1507 maize including cultivation. The monitoring plan has been developed (**Section 5** of notification **C/ES/01/01**) based on the conclusions obtained from the environmental risk assessment (e.r.a.) for the placing on the market of 1507 maize, which has been elaborated as a separate document (**Section 4** of notification **C/ES/01/01**) in accordance to Annex II of Directive 2001/18/EC. The monitoring plan will be applied following approval for the placing on the market of 1507 maize.

As summarized in **Point B.29.** above, the conclusions from the e.r.a. confirm that there is no risk to human and animal health or the environment arising from the placing on the market of 1507 maize. In addition, there is no significant risk to non-target organisms. However, the e.r.a. identified a limited potential risk posed by the cultivation of 1507 maize due to the potential development of resistance to CRY1F protein as expressed in 1507 maize within the target insect pest population. Therefore, and in order to ensure that placing on the market of 1507 maize poses negligible risk, appropriate monitoring and risk management plans have been developed and proposed in the context of product stewardship.

The case-specific monitoring plan for 1507 maize will form part of the Insect Resistance Management (IRM) proposal entitled 'Resistance management proposal for genetically modified insect-protected 1507 maize expressing the Cry1F insecticidal protein from *Bacillus thuringiensis* sbsp. *aizawai*' and described in **Annex 3** of the e.r.a (see **Section 4** of notification **C/ES/01/01**). The IRM proposal has been developed in order to maintain the efficacy of the CRY1F protein in 1507 maize, thereby sustaining the environmental benefits of the *Bacillus thuringiensis* (Bt) technology. It is based on the following five principles: *(i)* deploying products with an effective dose of Bt protein; *(ii)* maintaining adequate refuges; *(iii)* monitoring product performance; *(iv)* educating seed distributors and farmers; and, *(v)* continuing to conduct research.

In addition and in the framework of general surveillance, the notifiers will encourage growers to report any observed adverse effects on non-target arthropod populations. Subject to further discussion with the relevant national Competent Authorities and associated bodies, the co-ordination of a surveillance network, where appropriate and necessary, within the framework of their national routine surveillance programmes, for example the monitoring of agricultural cultivars or plant protection products, may also take place.