

February 28, 2006

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Comments on GM Potato Further Information

1. Please confirm that the GM potato lines that BASF propose to release are tetraploid?

All commercially cultivated potato varieties are tetraploid ($4n = 48$) (OECD, 1997). As the GM potato lines intended for the small-scale release are derived from commercial tetraploid potato varieties, these lines are tetraploid as well.

Comment: A karyotype (a microscopic picture of the potato chromosomes) should be available for the line being tested because that line has been tested elsewhere and it is unlikely that a karyotype has not been prepared .

3. Under the same paragraph it is stated: '*Plants potentially arising out of those seeds are usually weak, with poor agronomic performance and low competitiveness*'
Please supply information that supports this statement as in Ireland seed that originates from berries can survive and germinate and produce tubers under field conditions.

True potato seeds (TPS) can survive and germinate in the soil and the resultant potato plants might produce tubers. However, due to genetic segregation, the true seed derived plants differ from their parental plants. Their agronomic performance is poor and they show lower competitiveness. Further seed-derived plants show a lower early vigour as the nutritional resources in the seed are much lower than those in the tubers.

Comment: Some information of the segregation of the tightly linked Rpi-blb1 and Rpi-blb2 genes along with ahas gene in the potato volunteers arising from the fruits and seeds should be considered.

4. Page 11, it is stated '*There are no components of the vectors known to code for harmful substances*'.

Provide evidence to verify this statement.

The coding regions present on the T-DNA are those listed in the Table of genetic elements (see page 11), i.e. the *Rpi-blb2*, *Rpi-blb1* and the *ahas* genes. Based on database comparisons none of these genes are known to code for any harmful substances and none show similarity to known toxins or allergens.

Comment: A comparison of data bases for evidence of toxicity is necessary but not sufficient. The report of Prescott et al (J Agric. Food Chem. 2005 ,53,9023-30) showed that transgenes from bean inserted in pea produced a protein that caused inflammation (a potent immune response) in mammals. Inflammation is not considered in allergy (IgE responses)data bases so would likely be ignored because inflammation from plant proteins is not available in existing data bases. BASF does not appear to have considered such important concerns.

7. Page 15, 3 (a), it is stated: ‘In the genetically modified lines a low level of expression of both *Rpi-blb1* and *Rpi-blb2* has been demonstrated by real-time PCR analysis in leaves, stems, tubers and roots.

Do low levels of gene expression give tolerance to the blight fungus? Please clarify.

Yes, only low levels of gene expression of the inserted *Rpi-blb1* and *Rpi-blb2* genes are needed to confer resistance against *Phytophthora infestans*. The R-gene products exert no direct activity towards *Phytophthora infestans*. Instead, they are acting as “guards” that recognize pathogen-derived molecules and upon recognition initiate the resistance response including a localized cell death trapping the pathogen. For this recognition to take place only small amounts of R-gene products are required. NBS-LRR genes are usually expressed at low levels (Michelmore, 2000). Massively parallel signature sequencing (MPSS; Meyers et al., 2004) data of NBS-LRR proteins in Arabidopsis and rice obtained from Plant MPSS Databases (<http://mpss.udel.edu/>) further confirms low expression levels for many more such genes.

Further, the GM potato plants that express the inserted *Rpi-blb1* and *Rpi-blb2* genes at low levels are able to resist infection by *Phytophthora infestans* when challenged in the greenhouse.

Comment: The Rpi-blb genes are regulatory genes of the NBS-LRR family, as regulatory genes they may exert a strong response without producing a great deal of protein. However, resistance genes that produce too little product may contribute to selection of resistant pests. The development of resistant pests should have been studied in glasshouse rather than field experiments because the glass house provides a more controlled environment for such studies.

10. Page 16, No 7, *Information on any toxic, allergenic or harmful effects on human health and the environment arising from the genetic modification.*

Please indicate if the following studies have been addressed/ examined/taken into consideration. Where the studies have not been undertaken please explain why.

- **comparison of homology (bioinformatic database search) between amino acid sequence of all the inserted genes (blight and herbicide tolerance genes) and amino acid sequence of known toxic/allergenic proteins?**

The AHAS, *Rpi-blb1* and *Rpi-blb2* protein sequences were compared *in silico* to all proteins in a downloaded Food Allergy Research and Resource Program (FARRP) Allergen Protein Database (version 6.00) via a BlastP (version 2.2.6) analysis. None of the submitted proteins showed 35% or greater identity over 80 amino acids to a known allergen. Additionally, no submitted protein shared a sequence of eight consecutive identical amino acids with a known allergen. The AHAS, *Rpi-blb1* and *Rpi-blb2* proteins were also analyzed for sequence homology to known toxins via a BlastP search of the downloaded June 2005

GenBank non-redundant peptide sequence database. This BlastP analysis did not identify any known toxins with significant homology to the submitted proteins.

Comment: This matter has been considered under item 4. As stated above, sequence comparisons are necessary but not sufficient to establish safety. Particularly as inter-species plant gene transfers have led to effects such as inflammation in mammals consuming the modified plants. Inflammation has not been adequately included in the data bases used for comparison.

- **possible epigenetic or pleiotropic effects pertaining to the introduced DNA?**

Potential epigenetic or pleiotropic effects in plant development generally relate to heritable changes in gene expression that are not associated with alterations in DNA sequence. These effects can be exerted at transcription level or posttranscriptionally. Many epigenetic changes depend on the recognition of sequence homology at the DNA or RNA level. This recognition can lead to transcriptional or posttranscriptional gene silencing. None of the genetic elements present on the T-DNA to be integrated into the potato genome show sufficient DNA sequence homology to respective endogenous potato genes in order to be able to trigger any epigenetic or pleiotropic effects. Therefore the likelihood of the occurrence of possible epigenetic or pleiotropic effects in the GM potato lines pertaining to the introduced DNA is remote.

Comment: Definitions of the terms epigenetic or pleiotropic plei-ot-ro-pism also plei-ot-ro-py The control by a single gene of several distinct and seemingly unrelated phenotypic effects.

epigenetic effects - Changes in cellular biochemistry that influence the phenotype produced from a genotype. Epigenetic effects differ from genetic effects, which are caused by DNA mutation. The earliest modern use of the term was by Conrad Waddington in 1942, he defined it as the causal mechanisms of development.

It seems to me that the NBS-LRR class of regulators are bound to produce both pleiotropic and epigenetic impacts because they govern a range of responses leading to cell death. It seems like good common sense to take those impacts into account.

- **have any of the proposed 340 GM potato lines been measured for the main toxic or anti-nutritional substances (glycoalkaloids and nitrates) found in potatoes?**

None of the GM potato lines intended for the release have been analysed for tuber composition, i.e. glycoalkaloids or nitrates. The genetically modified potatoes differ from conventional potato varieties in their resistance to *Phytophthora infestans* conferred by the introduced R-genes. Potato already contains a large number of resistance genes conferring resistance against other plant diseases where the majority of those genes belong to the NBS-LRR class. Included are also genes introgressed from wild potato species. None of the genes are known to exert

any toxic or allergenic effects to human health. The mode of action is a hypersensitive response upon infection by the fungus leading to plant cell necrosis. The introduced genes are expressed by their endogenous promoters at very low levels that are comparable to those from other endogenous resistance genes.

Comment: As pointed out earlier, inter-specific gene transfer has led to the formation of a toxin causing inflammation in mammals, it seems unwise to ignore that finding and proceed based on blind supposition. Certainly the regulatory proteins are known to be immunologically active but these and toxic effects of the proteins from *S bulbocastanum* have not been studied adequately regarding their impact on mammals.

The introduced selection marker gene is expressed as the enzyme AHAS, which is an enzyme found in all plant species and not known to confer any toxic or allergenic properties. The safety of different crop species with AHAS-mediated tolerance to imidazolinones has been assessed by the Canadian Food Inspection Agency.

The potato plants are intended to be released within the scope of a small-scale field trial, are not for human consumption and measures taken with regard to planting, harvest, storage and transportation will minimize any contact to humans. Therefore the overall impact on human health is negligible.

Comment: The Canadian Food Inspection Agency reviewed a mutant ahas gene used to confer resistance to imidazolinones in spring wheat. That mutant was not a transgene but was a mutation selected in spring wheat. There is no real justification in implying that the mutation and its consequences are the same as the results of an inter-species transgene transfer between *Arabidopsis* (bearing a different mutation) and potato. At any rate the Canadian Agency assumed that mutant and natural ahas were substantially equivalent but did not do any experiments to support their superstition. BASF was wrong, I believe, not in mentioning the Canadian review but in failing to mention what ahas mutation had been studied in spring wheat.

Moreover we would like to point out that the release, if consent is granted, will be a small-scale experimental trial at one location to be conducted in two phases (A) screening of events (proof of concept; duration 1 to 2 years) and (B) development (stability of trait, safety studies; duration 2 to 3 years). During the course of the trial the following will be observed and recorded: Phase (A): agronomic performance (e.g. plant vigour, yield, susceptibility to climatic factors), altered agronomic properties (e.g. disease susceptibility), event screening (susceptibility to *Phytophthora infestans*), plant characteristics (e.g. emergence, leaf shape and colour, flowering, ground coverage, maturation), management of volunteers and Phase (B): stability of expression (e.g. sampling of plant tissue at various developmental stages in order to conduct gene expression studies), potential effects on non-target organisms (e.g. soil flora, potato-related insects), altered qualitative properties (e.g. tuber composition, nutrients, anti-nutrients, feeding studies).

Therefore the field trial will enable the generation of GM potato material grown under Irish field conditions for compositional analyses.

Comment: Have the results of such comprehensive studies been made available from the Swedish field trials?

- **Have animal feeding trials been carried out on any of these GM potato lines?**

No animal feeding trials have been carried out on any of the GM potato lines intended for the release. However, the field trial will enable the generation of sufficient GM potato material grown under field conditions in Ireland that may be used for animal feeding studies within the frame of safety studies (see point above). During the course of the trial measures taken under current release practice will protect the trial against damage by wild animals (e.g. fences) and also ensure that seed stock and plant material are harvested, stored, transported or disposed of (e.g. cleaning of machinery, packaging) in such a way to minimise contact to animals. Measures will also be taken in order to prevent that GM potatoes enter the food or feed chain.

Comment: Have animals feeding experiments been initiated or completed using material from the Swedish field trials? Those trials should have provided more than enough material for animal feeding studies and would have provided useful information about the impact of two of the three genes.

15. **19.** Page 20, F 1, *additionally, the release is aiming at, in the context of safety studies, collecting necessary data in comparison to unmodified recipient varieties and to conventional potato varieties. Data on agronomic properties and also on environmental interactions will be generated.*

Specify the safety studies and studies on environmental interactions you propose to carry out, if consent is granted?

If consent is granted the following studies are proposed to be carried out within the timeframe of 5 years (2006 to 2010) for selected GM potato lines. We would like to point out that the release, if consent is granted, will be a small-scale experimental trial at one location to be conducted in two phases (A) screening of events (proof of concept; duration 1 to 2 years) and (B) development (stability of trait, safety studies; duration 2 to 3 years). During the course of the trial the following will be observed and recorded: Phase (A): agronomic performance (e.g. plant vigour, yield, susceptibility to climatic factors), altered agronomic properties (e.g. disease susceptibility), event screening (susceptibility to *Phytophthora infestans*), plant characteristics (e.g. emergence, leaf shape and colour, flowering, ground coverage, maturation), management of volunteers and Phase (B): stability of expression (e.g. sampling of plant tissue at various developmental stages in to order to conduct gene expression studies), potential effects on non-target organisms (e.g. soil flora, potato-related insects), altered qualitative properties (e.g. tuber composition, nutrients, anti-nutrients, feeding studies).

Comment: Feeding studies should be well planned and laid out. Rodents should be fed and experiments concluded by full necropsy and tissue analysis by qualified veterinary pathologists.

16. 27. Page 26, H (vi), *None of the genes are known to exert any toxic or allergenic effects to human health.*

Also page 30, ERA, it is stated that *the potential effect on human or animal health due to introduced R-genes is negligible. NBS-LRR genes not known to confer toxic or allergenic properties and that the overall impact is negligible.*

Provide data to support these statements and explain how you concluded in your determination of the risk of the GMOs (in the ERA) that the overall impacts are negligible, if you cannot support these statements with relevant data/studies? See also question 10 above.

The Rpi-blb1 and Rpi-blb2 protein sequences were compared *in silico* to all proteins in a downloaded Food Allergy Research and Resource Program (FARRP) Allergen Protein Database (version 6.00) via a BlastP (version 2.2.6) analysis. None of the submitted proteins showed 35% or greater identity over 80 amino acids to a known allergen. Additionally, no submitted protein shared a sequence of eight consecutive identical amino acids with a known allergen.

The Rpi-blb1 and Rpi-blb2 proteins were also analyzed for sequence homology to known toxins via a BlastP search of the downloaded June 2005 GenBank non-redundant peptide sequence database. This BlastP analysis did not identify any known toxins with significant homology to the submitted proteins.

As NBS-LRR proteins in general are not known to be toxic or allergenic and as none of our introduced R-proteins shows any homology to known allergens or toxins we concluded in the e.r.a. that the overall impact to human or animal health is negligible.

Comment: As indicated earlier, the search of DNA sequence data is necessary but not sufficient to establish safety of transgenes. Even, interspecies plant gene transfers can lead to toxic products. In one reported case the plant to plant gene transfer led to protein modifications that cause immunotoxicity leading to inflammation. Common sense leads us to the need for direct animal feeding experiments for all of the transgenic crops, whether plant to plant, bacteria to plant, fungus to plant or human to plant.

31. 31. In the ERA part of the notification you are requested to comment on the following potential effect:

Please comment on whether the introduction of your GM blight resistant potatoes might alter the pathogenicity of the potato blight fungus under Irish soil conditions thus facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors.

The mechanism of response conferred to the genetically modified potatoes by the R-genes is one of hypersensitivity and very specific to the interaction of the host and the pathogen. R-genes encode receptors that will recognize specific elicitors injected by the pathogen into the plant cell. This recognition will

through a signalling network trigger both local and systemic defense responses. The local response aims at trapping the pathogen in the cells by localized cell death thus stopping further penetration and spreading.

Further the durability of the conferred resistance and therefore the lack of the pathogen to alter its pathogenicity is also supported by a large body of evidence. Schilde-Rentschler et al. (2002) produced somatic hybrids between *S. tuberosum* and *S. bulbocastanum*. The hybrid material containing the *blb1* gene has been tested in several countries (Germany, Spain, Bolivia, Columbia, Costa Rica and Ecuador). Furthermore, Helgeson et al. (1998) reported the production of somatic hybrids containing *blb1*. This material was tested at Hancock, Wisconsin and in Mexico for several years, whereas the progeny showed remarkable high levels of late blight resistance. Breeding clones containing *blb2* have shown high levels of resistance in experimental organically grown fields in the Netherlands from 1999 to 2002 (not published). The basic material from which those breeding clones were derived has been tested already in the eighties for resistance in Mexico (Hermsen, 1983) and proven highly resistant.

In addition to the evidence for the durability of the resistance, the genetically modified late-blight resistant potatoes are intended to be released into the environment within the frame of a small experimental field trial. Due to the scale (size, timing) of the trial as well as the presence of conventional potato varieties with a susceptibility to *P. infestans* the selection pressure exerted onto the oomycete will be negligible and the likelihood of a change in its pathogenicity is considered negligible.

Comment: The basic question is ‘how soon will the fungus grow resistant the transgenic potato?’ Appropriate glasshouse experiments should provide a useful estimate as to the time to resistance. Turning to the *S. bulbocastanum-S.tuberosum* somatic hybrids. I understand that the hybrids proved too unstable to produce commercial lines. If the lines were stable then there would not likely be any need for BASF to promote transgenic lines.

It is worth pointing out that there do not appear to be any published reports on the experiments where the somatic hybrid potatoes were fed to mammals in a laboratory situation. Since breeding clones of *blb2* have been grown in the Netherlands since 1999 it is surprising that no effort has been reported on the tests of such clones in animal feeding experiments. Of course, it would be negligent to withhold such studies, should they exist.

Final Comments

The BASF proposal to release transgenic potatoes to the Irish environment seems premature because fuller laboratory and glasshouse studies to insure the safety of the recombinant organisms. In particular, there was little or no reference to evidence that plant to plant gene transfer may lead to formation of proteins toxic to mammals.

