Notification C/SE/96/3501

Placing on the Market of the Amylopectin Potato Clone EH92-527-1

REPLY TO QUESTIONS RAISED BY THE EFSA GMO PANEL UNDER DIRECTIVE 2001/18/EC

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Introduction

Amylogene HB submitted in 1996 an application for placing on the market of a genetically modified higher plant according to Council Directive 90/220/EEC to the Swedish Competent Authority. The notification is now managed by Plant Science Sweden AB, a fully owned subsidiary of BASF Plant Science GmbH, and has been complemented according to Article 35 of Directive 2001/18/EC.

On April 8, 2004 the Swedish Competent Authority forwarded its Assessment Report and any other information on which it has based its report, to the Commission according to Article 14 § 2 of Directive 2001/18/EC. The assessment report indicated that the GMO in question should be placed on the market under certain conditions.

The Commission forwarded the notification to the Competent Authorities of the other Member States. Several Competent Authorities asked for further information, made comments or presented reasoned objections to the Placing on the Market of Potato Clone EH92-527-1 within a period of 60 days from the date of circulation of the assessment report. The notifier's reply was forwarded to the Member States in December 2004. Additional clarification on comments by Member States at the Commission's Technical Meeting on 11 January 2005 was forwarded to the Member States in January 2005.

Due to objections being maintained by Member States the Commission forwarded the notification to the EFSA GMO Panel on 10 February 2005 to seek an opinion. This document complements the notification C/SE/96/3501 and addresses questions raised by the EFSA GMO Panel Working Groups.

1. Response to questions raised by the Environmental Working Group

a. Does the environmental risk assessment allow for the cultivation of this potato in warmer parts of Europe where feral potatoes are more commonly found?

The environmental risk assessment for amylopectin starch potato EH92-527-1 follows the step wise approach identifying those characteristics of the event which may cause adverse effects, evaluating the potential consequences and the likelihood, estimating the risk, considering management strategies and determining the overall risk. Apart from the recipient organism and the genetic modification also characteristics like the methods traditionally employed to cultivate starch potatoes (identity preservation system, contract farming, control by developer and/or starch producer, agricultural management practices) as well as the regions in the EU where starch potatoes are traditionally cultivated (Eastern Europe, Nordic countries, Germany, Netherlands, France and Belgium) are being considered. The environmental risk assessment does not exclude particular regions solely based on their climatic conditions e.g. warmer parts of Europe.

Most of the data in support of environmental the risk assessment have been obtained in the multiple field trials conducted since 1993 in Sweden. Since 2004, trials are performed in the Netherlands as well as in Germany. All field trials have been conducted in regions where starch potatoes are typically grown. The characteristics of these regions like temperature, rain fall, soil types, disease and pests are also typical of other regions in Europe where starch potatoes are generally cultivated. Therefore the data is considered supportive of amylopectin potato event EH92-527-1 for cultivation in other important starch potato production areas of Europe.

The points of relevance in the e.r.a. regarding 'feral potatoes and warmer parts of Europe' are (i) the potential for gene transfer (plant to plant) and (ii) potential unintended effects on plant fitness due to the genetic modification.

The potential for gene transfer to related species e.g. potatoes within and outside cultivated areas was assessed and it was concluded that due to the biology of the mother potato variety and the derived event (propagation via tubers, limited reproduction via true seeds, self-fertilization, limited dispersal of pollen outside the field) and due to common agricultural management practices like treatment of volunteers, isolation and crop rotation the likelihood for successful gene transfer to related species was estimated to be negligible. The consequences of the gene transfer, should it occur, were also evaluated, and include the transfer of the trait gene (reduction of amylose and increase of amylopectin in the tuber) and marker gene (tolerance of plant cells to kanamycin, presence of APH(3')II protein). The consequences of a gene transfer to the environment, human and animal health were evaluated as negligible due to the insignificant addition of amylopectin or APH(3')II protein to an environment where those components are already abundant in starch containing crops (amylopectin) and soil microorganisms (APH(3')II protein) and the

absence of any selective advantage or disadvantage. Based on the likelihood and consequences of a successful gene transfer to potatoes within and outside (e.g. feral potatoes) of the agricultural environment the overall risk was estimated to be negligible.

The potential effects on plant fitness were assessed and it was concluded that due to potatoes being poorly competitive, failing to survive outside of cultivated fields and due to the unchanged sensitivity to pests, diseases and weed control practices relative to the mother starch potato variety the overall risk for EH92-527-1 to become more persistent in agricultural habitats or more invasive in natural habitats is negligible. Should EH92-527-1 be cultivated in warmer regions, then there is no indication that the plants will behave differently than the mother variety Prevalent. The risk to successfully produce progeny via true seeds with feral populations and/or to be come established as a feral plant outside of the agricultural environment is considered unchanged from the mother variety and therefore negligible.

b. Which data support the conclusion that there is no impact on potato associated organisms such as invertebrates, pathogens etc. (see for example page 198 of the application dossier)?

Support for the conclusion that there is no impact of the cultivation of amylopectin potato EH92-527-1 on potato-associated organisms is several fold based on (i) the e.r.a. and (ii) experiments performed in the frame of variety testing. In addition a survey of potato-associated organisms in field trials in 2004 is provided in this reply.

Potential changes in the interactions of amylopectin potato clone EH92-527-1 with non-target organisms have been addressed in the e.r.a. An e.r.a. is a stepwise process of evaluating the potential hazard, the likelihood of the hazard being realized and the consequences of the hazard being realized. In the first step the potential of the amylopectin potato clone to cause adverse effects to non-target organisms was evaluated and the following points taken under considerations:

- Based on the agronomic and compositional data provided in the notification the equivalence of EH92-527-1 to conventional starch potatoes was established except for the trait. All measured parameters were within the range accepted for conventional starch potatoes.
- The risk assessment therefore focuses on the incremental increase of amylopectin in the tuber starch from 75 to > 98 % and the very low abundance of the APH(3')II protein in leaf and tuber tissue. Amylopectin is contained in all starchy food and feed products and is a component of all starch containing crops. Amylopectin is present in or exposed to the environment via cultivation of conventional potato varieties or cereals (covering a range of seed amylopectin levels) without any adverse effects.
- The safety of the APH(3')II protein has been determined in various studies and was expressed in a series of opinions by regulatory authorities and scientific

committees. None of the safety studies have shown any indication of potential harm to humans, animals or the environment.

Thus the hazard potential of amylopectin potato clone EH92-527-1 to non-target organisms can be considered negligible. Any interaction with non-target organisms via exposure through cultivation of EH92-527-1 potatoes will be comparable to non-genetically modified potatoes, since none of the modifications will make the potato more or less attractive to insects, mammals or birds.

Further a statement is provided by SLU Jannie Hagman 'No evidence that the clone EH92-527-1 was more susceptible or more resistant to late blight (*Phytophthora infestans),* potato early blight (*Alternaria solani*), *Erwinia* rots, other bacterial diseases, potato cyst nematodes (*sp Globodera*), aphids or leafhoppers was found.' As stated on previous occasions the notifier has no access to the studies conducted during the variety testing by the SLU Department of Ecology and Crop Production Science.

Additional support is presented in a survey (***Annex 1*** to this document) on potato-associated arthropod populations conducted during field trials of EH92-527-1 potato in comparison to conventional starch potatoes in Sweden, Germany and the Netherlands in 2004. The survey used as a method pitfall traps to reflect the activity of arthropods, beat traps to monitor their abundance associated with the plants and yellow and blue sticky traps to monitor flying insects as they visited the field. The main conclusions of the survey are summarized as: (a) following pesticide application there were clear reductions in the number of insects associated with the potato plots, (b) the insect population recovered with time after treatment, (c) like most insects, the predatory and parasitoid taxa showed short-term reductions from the exposure to agrochemicals, however, no long term reductions were obvious, (d) there was no consistent long-term effect by the transgenic crop on any taxon analyzed and (e) short term effects identified were generally significant to one comparator line only, which suggests stronger differences between both comparator lines than between the GM line and either comparator line. In conclusion, there was no adverse effect of the amylopectin potato event EH92-527-1 on potato-associated organisms present in the field plots during the 2004 trials.

Given these confirmations, the notifier did not identify a need for additional case-specific studies regarding non-target organisms. Yet, the notifier committed to perform appropriate studies to determine the specific impact, in case at a later date indications would be observed on a potential impact on insects, mammals or birds through general surveillance or in case new scientific data suggest a potential impact of the introduced traits on these organisms. This is covered by obligations in the Directive 2001/18/EC for reporting new findings and provides the competent authorities with an immediate access to new information and with tools to react.

c. The applicant is asked to state more specifically how the IPS, i.e. form 5, can be used for the General Surveillance.

The Identity Preservation (IP) system constitutes an important management tool in order to preserve the unique characteristics and the value of amylopectin EH92-527-1 potatoes. Though the system is not a risk management tool and as such is not being anticipated for use as a monitoring tool, elements of the IP system allow recording at all stages of cultivation, accompanying measures and general observations. Within the IP system growers assume an important role in observing and channelling the crop. Due to their role and their familiarity with the crop they, as standard practice, continually monitor starch potatoes for changes in plant characteristics that may be significant to the performance of the crop as well as changes in the development and susceptibility to diseases and pests in the context of the general growing environment. At the same time the grower is best positioned to contribute with his observations and recordings to the items of the general surveillance like absence of differences in general plant characteristics, in disease and pest susceptibility and in competitive behaviour.

The IP system manual provides the grower with the appropriate tools not only with regard to training and support, but also to allow feedback via a set of forms. In this context the so-called field-plot card-index form 5 will fulfill two purposes first in securing the quality of the potato and identifying any deviations that would lead to an impact on quality and secondly serving as a tool that supports general surveillance. The use of field-plot card-index assures that relevant information on climate, soil, flora, fauna, plant and animal communities is captured via the subjects rain fall and weather, soil, fertilization and yield, weed control, parasites and nematodes, as well as treatment against Phytophthora infestans.

The field-plot card-index will be made available to all growers participating in the IP system for growing amylopectin starch potatoes EH92-527-1. All growers will be obliged, according to the instructions and training given, to record the requested data during the vegetation period and after harvest. The recording can either be performed manually or electronically and is submitted at multiple time points during cultivation and after harvest. The data generated based on the field-plot card-index are foreseen to be stored in a database, will be maintained per site and will be accessible to the notifier during and after the vegetation period and thus will be the most important tool to allow monitoring for any unanticipated or unforeseen effects of cultivating amylopectin potatoes in the agricultural environment which is the main objective of general surveillance.

The notifier will receive timely confirmation that observations according to form 5 have been performed including the results of the observations. In that way a rapid reaction is possible after a deviation has been observed and recorded. Any deviation as realized during the observations may provide an indication on unexpected effects, which on an as-needed basis could be followed-up by detailed studies to establish the cause of effect. However all observations are qualitative and not quantitative in

nature. If an evaluation of the differences observed leads to the conclusion that a qualitative finding would require further quantification, case specific studies can be established (in consultation with the competent authority) to provide scientifically reliable data. Finally reporting as outlined in the post-market monitoring plan will consist of a summary report once a year and ensure that if during the year an unexpected event occurs all involved competent authorities are informed of the unexpected event, the location and timing, as well as the initial evaluation of a potential change in impact and measures taken or planned to protect human health and the environment.

d. The applicant is asked to provide a separate farmer questionnaire, supplementing form 5, to allow farmers to record any unusual observations.

The farmer's questionnaire provided as part of the Identity Preservation System manual also called Field-plot card-index (Form 5 page 1 and 2) is designed to provide a means to record various factors like weather data and soil type, disease and pest pressure, soil and plant treatment, emergence, plant development and yield. Form 5 also allows recording of general observations with regard to those factors. The field-plot card-index has now been complemented by a third page in order to allow in more detail the recording of general as well as any unusual observations (***Annex 2*** to this document). Observations are performed based on the variety description for amylopectin potato EH92-527-1 (variety AMFLORA) and in general based on past experience of cultivating conventional starch potato varieties.

2. Response to questions raised by the Molecular Characterisation Working Group

a. Clear evidence should be provided to demonstrate the stability of the insert over several generations. In particular an explanation is needed for the apparent discrepancies in the information in Southern blots (eg data for EcoRI digestion hybridised with the *gbss* promoter probe).

The report provided previously on the 'Structure and DNA Sequence of Insert and Flanking Genomic Region of Potato Event EH92-527-1' was amended (***Annex 3*** of this document) to contain a Southern blot analysis addressing the question of genomic stability of the insert in potato event EH92-527-1. EH92-527-1 potatoes have undergone continuous rounds of vegetative clonal propagation since the initial identification of the event. In 1998 DNA was extracted from leaf tissue of plants that had undergone several rounds of propagation since 1992 and in 2005 DNA was extracted from leaf tissue of plants that had undergone further rounds of propagation since then. Both DNA samples were subjected to Southern blot analysis in parallel using the same combination of restriction enzymes and the same probe. The result provides clear evidence that the insert has remained genetically stable over several vegetative potato generations since the event was selected. The report has also been supplemented with an explanation on the apparent discrepancies in the case of a particular Southern blot analysis.

b. The applicant is requested to provide a more comprehensive bioinformatics analysis of the most recent insert DNA sequence data to identify any new ORF produced.

The attached ***Annex 4*** to this document provides a complete bioinformatics analysis of the insert in amylopectin potato event EH92-527-1. No new open reading frames other than those already outlined in Annex 8 to Notification C/SE/96/3501 Update were identified. The document complements the previously submitted report 'Bioinformatic Analysis of Potato Chromosomal Region Flanking the Insert of Potato Event EH92-527-1'.

3. Response to questions raised by the Food/Feed Safety Working Group

a. Concerning the 90-day rat study, the Panel would like to see the statistical comparison between the rat groups fed transgenic and non-transgenic potato since the applicant has only shown the results from the comparison of both diets with the standard diet.

The attached memo (***Annex 5*** of this document) supplements the 90-day rat feeding study with the requested statistical analyses comparing the rat group (Group 1) fed a diet of freeze-dried Prevalent potatoes and the rat group (Group 2) fed a diet of freeze-dried amylopectin EH92-527-1 potatoes. Regarding clinical examinations (food consumption, body weight data, food efficiency), clinical pathology (haematology, clinical chemistry, urine analysis) and pathology (absolute and relative organ weights) no overt significant changes were observed between both rat groups.

ANNEX 1

Nontarget Arthropods in Fields of Amylopectin Potato Event EH92-527-1 and Conventional Starch Potatoes in 2004.

ANNEX 2

Field-plot Card-index (Form 5).

ANNEX 3

Amended Report
Structure and DNA Sequence of Insert and Flanking Genomic Region of Potato Event EH92-527-1.

ANNEX 4

Bioinformatics Analysis of Open Reading Frames contained in the Insert of Potato Event EH92-527-1.

ANNEX 5

Additional Statistical Analyses
Genetically Modified Potato Meal
Subchronic toxicity study in Wistar rats
Administration via the diet over 3 months
(Project number: 50C0066/04012)