Determination of the Safety of Pioneer's and Dow Agro Sciences'
Combined trait product corn: 1507 x 59122
for Direct use as Food, Feed, and Processing

Food and Feed Safety:

The product dossiers on combined trait product corn: 1507 x 59122 were reviewed for safety and nutritional differences compared with the conventional corn. The focus of the food/feed safety assessment is based on three major issues/concerns regarding stacked genes from different sources namely a) gene interaction; b) effect on metabolic pathways and c) differential gene expression due to stacking.

A biosafety notification for combined trait product corn: 1507 x 59122 and all progenies derived from crosses of the product with any conventionally-bred corn and corn containing approved-biotech events for direct use as food, feed or for processing was issued to Pioneer Hi-Bred Inc. and Dow Agro Sciences of the Philippines on January 23, 2007. The notification is valid for five years and shall expire on January 22, 2012 subject to the terms and conditions set forth in DA Administrative Order No. 8, Series of 2002, and Memorandum Circulars Nos. 6 and 8, Series of 2004. The said combined trait product was included in the Lists of Approval Registry (Delisting) being prepared by the Department of Agriculture-Bureau of Plant Industry.

This approval is for use as Food, Feed and Processing only. This does not include cultivation of combined trait product corn: 1507 x 59122 in the Philippines. Food and Feed use of combined trait product corn: 1507 x 59122 its by-products is therefore authorized as of January 23, 2007. The biosafety notification (No. 07-014) stated that combined trait product corn: 1507 x 59122 is as safe for human food, livestock feed and for processing as its conventional counterparts".

I. Brief Identification of the Genetically Modified Organism (Living Modified Organism)

Designation: Combined trait product corn: 1507 x 59122

Applicant: **PIONEER HI-BRED INC. (PHI)**
24F Antel Global corporate Center
Dona Julia Vargas Avenue
Ortigas Center, Pasig City

and

DOW AGRO SCIENCES (DAS)
2nd Floor Bank of Commerce Building
J. Catolico Sr. Avenue, Lagao
General Santos City, South Cotabato

Plant Species:

Name: Corn *(Zea mays)*

Parent Material:	1507 x 59122 maize developed and produced by Pioneer Hi-Bred and Dow Agro Sciences
Center of Origin:	Mexico, Central America and South America
Toxic Factors/Allergen(s):	Trypsin inhibitor, phytic acid, and secondary metabolites such as raffinose, ferulic acid and p-coumaric acid are present in low amount 2-4 dihydroxy-7-methoxy-2H-1, 4 benzoxazin-3(4H)- one (DIMBOA) a potential toxicant but declines rapidly as the plant grows
Trait Description:	Insect resistance to certain lepidopteran and certain coleopteran insects
Trait Introduction Method:	Conventionally breeding
Donor Organisms:	*Bacillus thuringiensis* var. *aizawai* strain PS811 is the source of *cry1F* gene which confers resistance to lepidopteran insects. *Bacillus thuringiensis* strain PS149B is the source of *cry34Ab1* and *cry35Ab1* which confers resistance to corn rootworm *Streptomyces viridochromogenes* is the source of *pat* genes which confers tolerance to herbicidal active ingredient glufosinate-ammonium.
Pathogenicity:	*Bacillus thuringiensis* var. aizawai (PS811), the source of *cry1F* gene which is non-pathogenic to humans, plants and animals. Its toxicity is limited only to certain species of insects belonging to the Lepidopteran order. The Cry1F protein also does not possess or exhibit any allergic properties. *Bacillus thuringiensis* strain PS149B1, source of *Bt* genes *cry34Ab1* and *cry35Ab1*, produces insecticidal proteins that are very selective in toxicity to *Diabrotica* sp. Decades of safety testing on Bt proteins demonstrate the lack of toxicity to humans and animals, and the absence of adverse effects on non-target organisms and environment. *Streptomyces viridochromogenes*, the source of the *pat* gene, has no known adverse environmental or toxicological effects. All donor organisms have no known record toxicity, allergenicity or infectivity to human beings and animals.
Proposed Use:	For direct use as food, feed or for processing

II. Background Information

Pioneer Hi-Bred Inc. and Dow Agro Sciences of the Philippines have filed an application with attached technical dossiers to the Bureau of Plant Industry on August 25, 2006 for a biosafety notification for direct use as food, feed and for processing under Administrative Order (AO) No. 8 Part 5 for combined trait product corn: 1507 x 59122 which has been genetically modified for insect resistance and herbicide tolerance.

A safety assessment of combined trait product corn: 1507 x 59122 was conducted as per Department of Agriculture Administrative Order No. 8 Series of 2002. The focus of risk assessment is the gene interactions between the two transgenes**.**

Review of results of evaluation by the BPI Biotech Core Team in consultation with DA-Biotechnology Advisory Team (DA-BAT) completed the approval process.

III. Description of Novel (Introduced) Traits

The 1507 x 59122 maize has been obtained from traditional breeding methods between progeny of two genetically modified maize. The two GM maize events are DAS-59122-7 referred to as 59122 maize and DAS-Ø15Ø 7-1, referred to as 1507 maize. No new genetic modification has been introduced in 1507 x 59122 maize.

The 1507 maize has been genetically modified to express the proteins Cry1F and phosphinotricin –N-acetyltransferase (PAT). Expression of the Cry1F protein confers resistance against certain lepidopteran pests such as the European corn borer (*Ostrinia nubilalis*) and pink borer (*Sesamia* spp.), and expression of the PAT protein confers tolerance to the application of glufosinate-ammonium herbicide.

The 59122 maize expresses the Cry34Ab1 and Cry35Ab1 proteins which act together to control certain coleopteran insect pests such as corn rootworm larvae (*Diabrotica* spp.). In addition, 59122 maize expresses the PAT protein which confers tolerance to the herbicidal active ingredient glufosinate-ammonium.

Safety of the Expressed Proteins

The biochemical and molecular characteristics of the Cry1F. Cry34Ab1, Cry35Ab1 and PAT proteins, as shown in the previous individual applications of the parental lines 1507 and 59122, do not resemble the known characteristics of allergenic proteins. As shown in Figures 1-4, data in the form of electrophoretic gels of the Western blot procedure did not show any new proteins that could be a new allergen or toxin produced in the 1507 x 59122 maize.

Regarding the question on whether the gene products (Cry1F, Cry34Ab1, Cry35Ab1 and PAT proteins) will accumulate in the same or different sub-cellular compartments of the plant parts, the applicant responded that they Cry1F, Cry34Ab1 and PAT proteins were expressed in all tissues in 1507 x 59122 maize in all tissues in 1507 x 59122 maize plants and were not targeted to any specific organelle in individual cells. These proteins were detected in all tissue samples analyzed. In addition, western blots provided in the application confirm that the proteins produced in 1507 x 59122 were the expected size and weight and no additional proteins were detected as results of traditional breeding between progeny of 1507 and 59122 maize.

The mode of action of each of these proteins is expected to be the same in the 1507 x 59122 maize. The difference in the mode of action of each protein is expected to be as in the 1507 x 59122 maize. The products are not involved in the metabolic pathway. While Cry34Ab1 and Cry35Ab1 proteins act together to confer resistance to corn rootworm larvae (Cry1F and PAT proteins) are not part of the same metabolic pathway and therefore will not have any chance of being controlled by the same regulatory genes that would affect their production and cause any interaction.

The stacked genes involved function differently from each other. These genes have been combined through conventional breeding. There are no unexpe4cted effects of the stacked genes on the

metabolism of the plant because the procedure used is conventional breeding which has been practiced in many plants in general, in the long history of plant breeding.

Regarding the data from the protein expression experiments (ELISA technique) as shown in Table 1 which did not indicate whether a statistical test has been done to conclude that expression levels for each protein were similar between all maize lines analyzed. The data provided were collected from studies conducted in different locations and years and are not considered amenable for statistical analysis. Pioneer has since generated a new set of data in a greenhouse setting to show the Cry1F, Cry34Ab1, Cry35Ab1 and PAT protein expression across 1507 x 59122, 1507 and 59122 maize in multiple tissues sampled throughout the life cycle of the plant. The greenhouse maintained growing conditions typical of maize and three (3) representative samples were collected for each tissue.

No statistical difference were noted in respect to leaf, stalk, whole plant and grains matrices in the Cry1F, Cry34Ab1, and Cry35Ab1 protein expression after the observed probability was adjusted using the false recovery rate (FDR). Additionally, no statistical significant were noted in respect to stalk, forage and whole plant matrices in the PAT protein expression analysis after the observed probability was adjusted using the FDR. Significant differences were observed in the level of PAT in the leaf of maize line 59122 and 1507 x 59122 but were not unexpected due to the presence of 2 copies of the *pat* gene in the stacked product.

Again, in concluding that "all proteins within the combined trait product are expressed at similar levels" the analysis of variance was conducted to evaluate the difference between the desired treatments. Response variables were modeled by the fixed effect of the protein expression. The FDR method was used to account for the numerous comparisons between growth stages and tissues and minimize the number of differences being declared to be significant due to chance (PROC MULTTEST, SAS® version 9.1 software, SAS Institute Inc., Cary NC, USA). This issue is referred to in statistics as multiplicity. Controlling the FDR using FDR methods is preferable to controlling the experiment-wise error rate because more power is retained for detecting true differences among treatments.

The marker gene for the Pat protein has been shown by the data to be present or expressed in the 1507 x 59122 maize. There is no problem with this; since as marker gene, this should really be transferred and expressed too together with the genes of interest.

Data from the experiments conducted (western blot analysis) show that no new proteins were produced in the 1507 x 59122 maize. Each gene for each protein (Cry1F, Cry34Ab1, Cry35Ab1 and PAT) was expressed in both the parentals and the hybrid. Since no interaction is expected, the stability and expression level of either one of the genes should not be affected.

IV. Nutritional Composition (Compositional Analysis)

The World Health Organization (1995) stated that two plants that are substantially equivalent to conventional varieties are crosses by conventional breeding techniques, the combined trait product is expected to be substantially equivalent to the single event products. In accordance with OECD guideline (OECD, 2002) substantial equivalence was evaluated by comparing a) mean analyte values of the test maize to an appropriate control of similar genetic background, and b) mean proximate values of the test maize entry to analyte ranges available in the published literature.

V. Anti-Nutritional Factors

No known anti nutritional factors for individual events. Thus, 1507 x 59122 maize has no known antinutritional factors.

VI. Regulatory Decision

After reviewing the scientific data and information relevant to the combined trait corn 1507 x 59122 application of Pioneer Hi-Bred Inc. and Dow Agro Sciences of the Philippines, it is concluded that no interaction found between/among the combined traits, hence this plant product was found to be as safe as its conventional corn and can substitute for its traditional counterpart for direct use as food, feed and for processing and is therefore approved for direct use as food, or feed or for processing. Pioneer Hi-Bred and Dow Agro Sciences are hereby notified that it may proceed with the activities for the above product for direct use as food and feed or for processing following all existing rules and regulations consistent with DA AO #8.