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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Experimental Use Permit Application for Corn Expressing δ-endotoxin from Bacillus thuringiensis var. kurstaki from Ciba Seeds

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THIS REVIEW CONTAINS CONFIDENTIAL BUSINESS INFORMATION

DATA REVIEW RECORD

Active Ingredient:	δ-endotoxin from Bacillus thuringiensis var. kurstaki
ID No:	066736-EUP-R
Submission No:	S434296
MRID No:	426362-01- Summary of Product Chemistry 426362-02- Summary of Toxicology Data 426362-03- Summary of Residue and Environmental Data
Chemical No:	006430 Bacillus thuringiensis δ-endotoxin
DP Barcode:	D187330

ACTION REQUESTED

SAB was asked to review the submitted data to determine if it was adequate to support an experimental use permit (EUP) for the transgenic corn.

BACKGROUND

Ciba Seeds has submitted a request to field test transgenic corn expressing a truncated form of the δ-endotoxin from Bacillus thuringiensis var. kurstaki (Btk). The DNA to provide this trait has been introduced in 2 different forms reviewed below. This truncated form of the δ-endotoxin is similar to that found in

previous CIBA-Geigy submissions for use in tobacco. The company is currently field testing their corn varieties under an APHIS permit but expects to exceed the 10 acre limit requiring an EUP by July of this year. The first year of the EUP is expected to be for 33 acres. The requested second year of the EUP is for about 100 acres.

The following review focuses on the DNA construction of the transgenic corn plants so the Environmental Fate and Groundwater Branch (EFGWB) can appropriately evaluate the issue of containment. For the current SAB review, containment refers to the limitation of the pesticidal plant products to the transgenic plant on the test site. The rationale for the limited review is the lack of human exposure to the pesticidal products in a small-scale field test. The company has presented a limited toxicology summary and specifically requested no temporary tolerance determination for this EUP since they are proposing to destroy all the crop and residues. If EFGWB determines that this test is not adequately contained, SAB should be notified for further consideration of any possible toxicology data requirements for the EUP.

CONCLUSIONS

The current request for an EUP is adequate considering there are few human health effects possible with the limited exposure associated with an experiment run on a crop destruct basis and assuming EFGWB determine the study is contained.

PRODUCT CHEMISTRY & IDENTITY

The company has provided information relating to the transformation of their transgenic corn lines as well as a description of the introduced genes. Most of this information was found in Volume 2: Summary of Product Chemistry Data, MRID No. 426362-01.

HOST PLANT AND TRANSFORMATION

The sole corn line described as being transformed in this submission is CG00526 an inbred elite derived from Lancaster parentage. The 2 transformation events (171 & 176) were carried out by microprojectile bombardment of immature embryos (14-15 days after pollination). The resulting tissue was regenerated on a standard embryo culture media within phosphinothricin (PPT), a herbicidal selection agent that only transformed tissue would be resistant to. Subsequently the regenerated plants were also assayed for the presence of PPT resistance, GUS activity (only plants from transformation 171) and PCR analysis for certain sequences in the 35S promoter and the synthetic CryIA(b) gene. The regenerated plants were subsequently tested for expression of truncated Btk endotoxin by ELISA and activity against European corn borer as well as being crossed with CG00526 and other elite corn lines.

DESCRIPTION OF DNA CONSTRUCTS USED IN TRANSFORMATION

A mixture of plasmid DNA was used to coat the gold microprojectiles used for embryo bombardment in each event. Each mixture is described under the transformation event number.

Transformation 171 (mixture of pCIB4418, pCIB3064 and pCIB3007)

pCIB44118

Active Ingredient- ~2Kb of Cry IA(b) DNA coding for a C-terminal truncated version of the δ-endotoxin from Btk HD-1. The N-terminal 648 amino acids out of 1155 amino acids for the native protoxin are produced with an identical aa sequence. The DNA codons have been altered to yield ~65% G+C content which is more amenable for expression in corn. The company has also introduced ~100 bp of DNA from intron #9 for the corn phosphoenolpyruvate carboxylase (PEPC) gene after the CryIA(b) gene.

Promoter- 1 Kb of DNA from the 35S promoter region of CaMV

Terminator- 160 bp from CaMV containing the poly A site

pCIB3064

Marker Function- 0.6Kb of DNA from Streptomyces hygroscopicus coding for the enzyme phosphinothricin acetyltransferase

(PAT) termed the "bar gene". This gene is considered a marker gene for introduction of the pesticidal trait.

Promoter- 1Kb of DNA from the 35S promoter region of CaMV

Terminator- 160bp from CaMV containing the poly A site

pCIB3007

Marker Function- 1.8Kb of DNA from E. coli coding for the β-glucuronidase (GUS) gene. This gene is considered a marker for introduction of the pesticidal trait.

Promoter- 1Kb of DNA coding for the 35S promoter from CaMV and also containing 5' from the promoter the untranslated intron #1 from corn alcohol dehydrogenase (presumably ~535bp) and a 144bp sequence from CaMV

Terminator- 160bp from CaMV containing the poly A site

Transformation 176 (mixture of pCIB4431 and pCIB3064)

pCIB4431

Active Ingredient- ~2Kb of Cry IA(b) DNA coding for a C-terminal truncated version of the δ-endotoxin from Btk HD-1. The N-terminal 648 amino acids out of 1155 amino acids for the native protoxin are produced with an identical aa sequence. The DNA codons have been altered to yield ~65% G+C content which is more amenable for expression in corn. The company has also introduced ~100 bp of DNA from intron #9 for the corn phosphoenolpyruvate carboxylase (PEPC) gene after the CryIA(b) gene. Two copies of the endotoxin gene are inserted, each under the control of the 1 of the 2 different promoters described below.

Promoters- The first is a 2.3Kb of DNA from the promoter region of the corn PEPC gene. The second promoter is described as a 1.5Kb fragment from a pollen-specific gene in corn.

Terminator- For each copy of the Btk toxin gene described above the termination sequence is a 160bp poly A region from CaMV.

pCIB3064

Marker Function- 0.6Kb of DNA from Streptomyces hygroscopicus coding

for the enzyme phosphinothricin acetyltransferase (PAT) termed the "bar gene". This gene is considered a marker gene for introduction of the pesticidal trait.

Promoter- 1Kb of DNA from the 35S promoter region of CaMV

Terminator- 160bp from CaMV containing the poly A site

The company indicates that these traits are stably integrated into the corn genome. Transformants from both events display a 1:1 segregation ratio typical of Mendelian genetics for PPT resistance, CryIA(b) expression and European corn borer resistance when crossed with other elite lines of corn. Along with Southern hybridization analysis for the CryIA(b) gene alone, these data are said to indicate stable incorporation of the traits "at a single site of insertion, with a few copies of each gene present."

SAB COMMENTS

SAB would like to know the nature of the pollen-specific promoter used to drive one of the CryIA(b) genes especially for final registration purposes. There were no data presented to indicate that the traits are segregating by Mendelian genetics or integrated at a single site as claimed.

As a matter of interest, SAB would like to know:

Do the traits segregate independently implying integration events on separate chromosomes or appear to be linked on 1 chromosome?

Since these gene inserts lack the Agrobacterium TI border regions and the bar gene lacks even regions of homology with corn for recombination, what is probable method of integration for these introduced traits?

Does every bar^+ selected transformant express all the traits of the plasmid mixture?

EXPRESSION LEVELS OF BTK ENDOTOXIN IN CORN

The company has provided some information regarding the levels of Btk δ-endotoxin expressed in different tissues of hemizygous lines from their transformed corn embryo cultures. The data table found in volume 4: Summary of Residue and Environmental Data (MRID No. 426362-03) is also presented in a paper (M.Koziel et al., 1993, in press) accompanying the submission.

In short, the Btk endotoxin levels (by ELISA) range from not detectable by ELISA in the pollen/anther tissue to 4381ng/mg protein in pith tissue for transformation 171 and from 15ng/mg protein in the kernel to 1842ng/mg protein in leaf tissue for transformation 176. Transformation 171 represents Btk endotoxin expression driven by a 35S CaMV constitutive promoter and 176 has leaf and pollen tissue specific promoters. The tissues examined (leaf, root, pith, pollen/anther and kernel) had a great range of

expression values but it is difficult to evaluate much of the data except the leaf values due to a lack of replication. In general, the Btk levels found in 35S CaMV transformants was high and consistent in the tissues tested except for pollen/anthers. In leaf/pollen specific transformants the Btk levels in leaves and pollen were high but the levels in other tissues were low.

SAB COMMENTS

The ELISA derived results provided for Btk expression levels in plants found in Volume 4 are not clearly related to plants under the EUP. The figures adequately describe an expression range for human health issues given that this submission is a crop destruct EUP. The results do indicate the range of expression expected for the trait in leaves, but not enough attempts at replications are available to determine that range in kernels. At registration, the company should be ready to state more accurate expression figures for the lines they expect to market, especially an upper limit of expression and the amount found in corn kernels and corn silage. These data may aid in a determination of active ingredient exposure for humans and domestic animals for food tolerance considerations.

SUMMARY OF TOXICOLOGY DATA

The basic argument presented in this section (Vol.3: Summary of Toxicology Data, MRID No. 426362-02) is that there are no reasonable concerns for human and domestic animal toxicity due to the minute amounts of CryIA(b) protein present and its specific toxicity. This specificity of the Btk δ-endotoxin is due to: 1) solubilization and protease cleavage of the protoxin in the alkaline midgut of target insects and 2) the specificity of the activated toxin for the midgut epithelium receptors of target insects. It is unclear if any further protease processing occurs with the truncated form expressed in these plants, so the basis of low toxicity rests solely on the argument of target midgut receptor specificity. This is, however, a hypothetical argument as the company has requested that this be a crop destruct EUP and therefore not asked for a tolerance determination at this time.

SAB COMMENTS

SAB believes it would be prudent for the company to proceed with a testing program to demonstrate the lack of toxicity of the truncated form of the Btk CryIA(b) endotoxin to justify a tolerance exemption. Ideally the substance used for this testing should be derived from the transgenic plant. In lieu of this, the substance could be produced in an alternate system such as a bacterium or yeast to provide adequate quantities for testing at a maximum hazard dose.

The company may also want to demonstrate equivalency of their truncated Btk endotoxin product with naturally obtained protease digested CryIA(b) protoxin either by aa sequencing analysis and/or similar migration by PAGE or isoelectric focusing. This would add weight to a presentation of the DNA codon changes indicating neutral changes with increased G+C content.