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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

FEB 1 1 2009

MEMORANDUM

SUBJECT: Review of Supplemental Data in response to data deficiencies noted for the combined Plant-Incorporated Protectant products: Bt11 x MIR162 maize hybrid [EPA Reg. No. 67979-RE] and Bt11 x MIR162 x MIR604 maize hybrid [EPA Reg. No. 67979-RG] in support for Sec. 3 Registration, submitted by Syngenta Seeds, Inc. – Field Crops- NAFTA

FROM: Annabel Waggoner, Environmental Protection Specialist
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THROUGH: John L. Kough, Ph.D., Senior Scientist
Microbial Pesticides Branch, Biopesticides and
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TO: Jeannine Kausch, Regulatory Action Leader
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511P)

ACTION REQUESTED: To review the supplemental data submitted in response to the data deficiencies noted in the characterization of TRYCRY1AB-0105 (MRID No. 471372-11), for use as a test substance surrogate for use in ecotoxicity studies. These data were submitted to support the registration of PIP stacked products- Bt11 x MIR162 maize hybrid and Bt11 x MIR162 x MIR604 maize hybrid, crossed via traditional breeding methods. The registered *Bacillus thuringiensis* (*Bt*) derived PIP Corn Events were: Cry1Ab protein as expressed in Event Bt11; Vip3Aa20 protein as expressed in Event MIR162; and/or modified Cry3A protein as expressed in Event MIR604.

CONCLUSION: The data demonstrate the equivalence between the plant- and microbial-produced Cry1Ab proteins in support for utilizing the TRYCRY1AB-0105, as a test material in the non-target organism studies for demonstration of lack of synergism in the stacked Bt11 x MIR162 and Bt11 x MIR162 x MIR604 PIP products. Therefore, the outstanding data was addressed by the registrant and the data are acceptable to support the Sec. 3 registration of the combined PIP products: Bt11 x MIR162 and Bt11 x MIR162 x MIR604 maize hybrids.

THIS REVIEW DOES NOT CONTAIN CONFIDENTIAL BUSINESS INFORMATION

DATA REVIEW RECORD:

Active Ingredients: 1) Plant-Incorporated Protectant (PIP) *Bacillus thuringiensis* (*Bt*) Cry1Ab protein as expressed in Event Bt11 and the genetic material (*via* elements pZ01502) necessary for its production [OECD Unique ID. SYN-BTØ11-1],

2) PIP *Bt* Vip3Aa20 protein as expressed in Event MIR162 and the genetic material (*via* elements pZNOV1300) necessary for its production [OECD Unique ID. SYN-IR162-4], and/or

3) PIP *Bt* Modified Cry3A protein as expressed in Event MIR604 and the genetic material (*via* elements pZM26) necessary for its production [OECD Unique ID. SYN-IR6Ø4-8]

Product Names: *Agrisure* ™ 2100, Bt11 x MIR162 hybrid [EPA Reg. No. 67979-RE] and *Agrisure* ™ 3100, Bt11 x MIR162 x MIR604 hybrid [EPA Reg. No. 67979-RG]

Company Name: Syngenta Seeds, Inc. – Field Corps- NAFTA

ID No: 67979

Chemical Number: 006461, 006599, and/or 006509

Decision Number: 379488 and 379490

DP Barcode: 359322 and 359309

MRID No:

476049-01 Response to Data Deficiencies Noted for the Bt11 x MIR162 and Bt11 x MIR162 x MIR604 Applications for Registration [EPA Reg. No. 67979-RE and 67979-RG]

BACKGROUND:

On May 17, 2007, Syngenta Seeds, Inc. – Field Crops – NAFTA (Syngenta) submitted applications for registration of the plant-incorporated protectants in Bt11 x MIR162 corn [EPA Reg No. 67979-RE] and Bt11 x MIR162 x MIR604 corn [EPA Reg. No. 67979-RG]. These combined-trait products were produced by crossing the individual events: Bt11, MIR162, and MIR604 (via conventional breeding). Both combined-trait corn products express the Cry1Ab and Vip3Aa20 insecticidal proteins. Bt11 x MIR162 x MIR604 corn alsoexpresses the mCry3A insecticidal protein. Vip3Aa and Cry1Ab proteins are intended to control several lepidopteran pests of corn including: *Agropis ipsilon* (black cutworm), *Spodoptera frugiperda* (fall armyworm), *Spodoptera exiguai* (beet armyworm), *Heliothis virescens* (tobacco budworm), and *Helicoverpa zea* (corn earworm). Modified Cry3A protein will provide control for coleopteran pests of corn including: *Diabrotica virgifera vigifera* (western corn rootworm) and *Diabrotica longicornis barberi* (northern corn rootworm).

In 1996, EPA granted a registration for the *Bt* subsp. *kurstaki* strain HD-1 Cry1Ab protein and the genetic material necessary for its production in Event MON 810 corn [EPA Reg. No. 524-489]. The Agency concluded that there were no adverse effects on human health from the use of the Cry1Ab

protein expressed in corn. Therefore, an exemption from the requirement for a food tolerance was established when Cry1Ab protein is used as a plant-incorporated protectant [40 CFR § 174.511]. Syngenta bridged safety data from MON810 and provided additional product characterization data to register Cry1Ab expressed in Event Bt11 corn. The PAT protein is also expressed with Cry1Ab in Bt11 corn, as a PIP inert ingredient, and has an exemption from the requirement of a tolerance in all plant raw agricultural commodities [40 CFR § 174.522].

The product characterization, toxicological, and allergenicity data supporting the registration of *Bt* Cry1Ab delta-endotoxin and the genetic material necessary for its production in corn (plasmid vector pZO1502), are found in the 2001 *Bt* Crops Reassessment. EPA determined that the human health data previously submitted for Cry1Ab produced in MON810 is applicable to the Cry1Ab produced in Event Bt11 (US EPA, 2001).

Event MIR162 maize, was produced by *Agrobacterium*-mediated transformation using elements of a vector (pNOV1300) containing a variant of the *vip3Aa1* gene, which was isolated from *B. thuringiensis* strain AB88. This gene encodes a vegetatative insecticidal protein Vip3Aa20 that is highly toxic to several lepidopteran pests of maize. This protein is 99.8% identical to another Vip3Aa variant, (Vip3Aa19 as expressed in COT102 cotton) another registered PIP product. The product characterization, toxicological, and allergenicity data supporting the registration of *Bt* Vip3Aa19 protein expressed in COT102 and the genetic material necessary for their production in cotton, are found in the VipCot BRAD (US EPA, 2008). The Agency established a permanent exemption from the requirement of a tolerance for Vip3Aa proteins in cotton and corn [40 CFR § 174.505], as part of the Sec. 3 registration of Event COT102 cotton.

Event COT102 cotton and Event MIR162 maize also contain the *manA* gene from *Escherichia coli*, which encodes the selectable marker, phosphomannose isomerase (PMI). A permanent exemption from the requirement of a tolerance was established for PMI in all crops when used as a PIP inert ingredient [40 CFR § 174.527]. The product characterization, toxicological, and allergenicity data to support the registration of *Bt* Vip3Aa20 protein in event MIR162 corn [EPA Reg. No. 67979-14] were reviewed by the Agency and registered as a PIP on November 26, 2008 [see EPA memorandum from I. Barsoum, Ph.D. to J. Kausch, dated September 11, 2008].

The other protein expressed in the combined trait PIP product- Bt11 x MIR162 x MIR604 is modified Cry3A in Event MIR604. Syngenta's Event MIR604 corn was transformed with the synthetic *cry3A* gene to express modified Cry3A protein and provide resistance to western corn rootworm and northern corn rootworm. The Agency established a permanent exemption from the requirement of a tolerance for Modified Cry3A protein in corn [40 CFR 174.505]. In addition, EPA issued a Sec. 3 Registration for *Bt* mCry3A protein and the genetic material necessary for their production (via plasmid pZM26) in Event MIR604 corn. The product characterization, toxicological, and allergenicity data supporting the registration of *Bacillus thuringiensis* mCry3A protein and the genetic material necessary for its production in corn (plasmid vector pZM26) are found in the mCry3A BRAD (US EPA, 2007).

Syngenta Seeds, Inc. also submitted data in support for two FIFRA Section 3 registrations- Bt11 x MIR162 hybrid, expressing containing *Bt* Cry1Ab and Vip3Aa in conjunction with the Bt11 x MIR162 x MIR604 hybrid, containing *Bt* Cry1Ab, Vip3Aa, and mCry3A proteins and the genetic material necessary for their production, respectively. The confirmation of molecular identity and protein expression levels for Bt11 x MIR162 corn hybrid and Bt11 x MIR162 x MIR604 corn

hybrid were reviewed and found acceptable by the Agency [see EPA memorandum A. Waggoner to J. Kausch, dated Oct. 31, 2008].

In support of the applications for registration of these two combined-trait products, a test substance characterization report (Kramer and Graser 2007; MRID No. 47137211) was provided for the microbially produced TRYCRY1AB-0105 test substance. The TRYCRY1AB-0105 test substance was used as the source of Cry1Ab protein for two nontarget organism studies submitted in support of the two new product applications. These nontarget organism studies were submitted specifically to address the potential for synergistic interactions between the insecticidal proteins:

- MRID No. 47137208; Laboratory study to determine the combined effects of Cry1Ab, Vip3Aa20 and mCry3A on the predatory beetle *Coleomegilla maculata*. Study No. 1781.6671.

- MRID No. 47153005; A laboratory study to determine effects of Vip3Aa20 + Cry1Ab + mCry3A proteins on the rove beetle *Aleochara bilineata* (Coleoptera: Staphylinidae). Study No. T002321-06-REG.

The Agency noted several data deficiencies in the TRYCRY1AB-0105 test substance characterization report and these studies were rated supplemental [see EPA memorandum: from A. Waggoner, through Z. Vaituzis, Ph.D., to J. Kausch, dated Oct. 31, 2008]. Deficiences included: no direct comparison to establish the identity of the microbially produced Cry1Ab test substance (TRYCRY1AB-0105) to the Cry1Ab protein produced in Event Bt11 corn. Therefore, the Agency requested: (1) a clearer image of the Western blot that shows the presence of the intact, biologically active Cry1Ab protein (66.3 kDa) from Bt11 corn; and (2) the mass spectral data to establish the two peaks corresponding to the Cry1Ab species (65.8 and 66.3 kDa) claimed to be present in the TRYCRY1AB-0105 test substance. These data were needed to support using the TRYCRY1AB-0105 test material in two non-target organism studies submitted to address the lack of synergism when Cry1Ab, Vip3Aa20, and mCry3A are combined in the stacked PIP products- Bt11 x MIR162 and Bt11 x MIR162 x MIR604.

Syngenta submitted a report summarizing the data for the equivalency between the microbially produced Cry1Ab test substance (TRYCRY1AB-0105) and Cry1Ab protein produced in Event Bt11 corn. The data reviewed in this report address these product characterization deficiencies. The additional data supporting the lack of synergy among the proteins produced in the two combined PIP products are reviewed in the environmental risk assessment of Bt11 x MIR162 and Bt11 x MIR162 x MIR604 combined PIP products (Waggoner and Vaituzis, 2009).

RECOMMENDATION:

The data to support the characterization of the Cry1Ab test material, TRYCRY1AB-0105, is upgraded to **ACCEPTABLE** (MRID No. 471372-11), when combined with the summary of data presented in MRID No. 476049-01. These data demonstrate the equivalence of the plant- and microbial-produced Cry1Ab proteins and support the use of TRYCRY1AB-0105 as a test material in the non-target organism synergism studies for the stacked Bt11 x MIR162 and Bt11 x MIR162 x MIR604 PIP products. Therefore, the data support the Sec. 3 registration of the combined PIP products: Bt11 x MIR162 and Bt11 x MIR162 x MIR604 hybrids. Toxicological and allergenicity data on the individual proteins (Vip3Aa, Cry1Ab, and mCry3A) support the finding that there is a reasonable certainty of no harm to the U.S. population, including infants and children through

exposure to these proteins in combined PIP products (Bt11 x MIR162 and Bt11 x MIR162 x MIR604). This includes all anticipated dietary exposures and all other exposures for which there is reliable information. Furthermore, the existing exemptions from the requirement of a food tolerance for Cry1Ab, Vip3Aa20, mCry3A insecticidal proteins support the use of Bt11 x MIR162 and the Bt11 x MIR162 x MIR604 corn products, when used as plant-incorporated protectants, respectively.

SUMMARY OF DATA SUBMITTED:

A summary of the data supporting the equivalency between the microbially produced Cry1Ab test substance (TRYCRY1AB-0105) and Cry1Ab protein produced in Event Bt11 corn are provided below.

MRID No. 476049-01 Response to Data Deficiencies Noted for the Bt11 x MIR162 and Bt11 x MIR162 x MIR604 Applications for Registration [EPA Reg. No. 67979-RE and 67979-RG]

The registrant submitted the mass spectra and a clearer reproduction of the Western blot gel as visual confirmation for establishing the molecular weight of the test substance TRYCRY1AB-0105, containing the *ca.* 66 kDa truncated form of the full length (*ca.* 130 kDa) Cry1Ab insecticidal protein. Total mass analysis of the Cry1Ab in test substance TRYCRY1AB found two predominant comigrating Cry1Ab species, with molecular weights of 66.3 and 65.8 kDa. Other supporting data include: insect bioassays demonstrating similar bioactivity of the test substance against ECB; N-terminal amino acid sequencing analysis of TRYCRY1AB-0105 in comparison to Cry1Ab protein expressed in Event Bt11; and peptide mass analysis via Q-TOF comparing the masses of individual peptides resulting from proteolytic digestion of a test sample to the masses of known peptides in a database. In addition, field trial results comparing the efficacy of the single PIP events (Bt11, MIR162, and MIR604) to the combination PIP products: Bt11 x MIR162 and Bt11 x MIR162 x MIR604, showed no enhanced toxicity among various target pest species. Collectively, these data demonstrated the equivalence between the plant- and microbial-produced Cry1Ab proteins in support of utilizing the TRYCRY1AB-0105 test substance, as a suitable surrogate for the non-target organism toxicity studies submitted in support for the registration of the stacked Bt11 x MIR162 and Bt11 x MIR162 x MIR604 PIP products.

CLASSIFICATION: This report is rated as **ACCEPTABLE.** In addition, the classification of MRID No. 471372-11 is upgraded from supplemental to **ACCEPTABLE**, when combined with the results of this report.

REFERENCES:

Dively, G.P. (2005) Impact of transgenic VIP3A x Cry1Ab lepidopteran-resistant field corn on the nontarget arthropod community. *Environmental Entomology* 34, 1267-1291. MRID 46784601

Lee, M.K., F.S. Walters, H. Hart, N. Palekar and J-S Chen. (2003) The mode of action of the

Bacillus thuringiensis vegetative insecticidal protein Vip3A differs from that of Cry1Ab δ-endotoxin. *App. Environ. Micro.* 69(8): 4648-4657.

Marcon, P.C.R.G., L. J. Young, K. L. Steffey, and B. D. Siegfried. (1999) Baseline susceptibility of European corn borer (Lepidoptera: Crambidae) to *Bacillus thuringiensis* toxins. J. *Econ. Entomol.* 92: 279 - 285.

US EPA. (2001) "Biopesticides Registration Action Document for the *Bacillus thuringiensis* (*Bt*) Plant-Incorporated Protectants," dated October 15, 2001.

US EPA. (2007) "Biopesticides Registration Action Document for the *Bacillus thuringiensis* (*Bt*) Plant-Incorporated Protectant Modified Cry3A Protein and the Genetic Material Necessary for its Production (via Elements of pZM26) in Event MIR604 Corn SYN-IR6 Ø4-8," dated March, 2007.

US EPA. (2008) "Biopesticides Registration Action Document for *Bacillus thuringiensis* modified Cry1Ab (SYN-IR67B-1) and Vip3Aa19 (SYN-IR102-7) insecticidal proteins and the genetic material necessary for their production in COT102 X COT67B cotton," dated June 26, 2008.

DATA EVALUATION RECORD

Primary Reviewer: Annabel Waggoner, Environmental Protection Specialist, BPPD

Secondary Reviewer: John L. Kough, Ph.D., Senior Scientist, BPPD

STUDY TYPE:	Product Identity (OPPTS Guideline No. 885.1100)
MRID NO:	476049-01
DECISION NO:	379488 and 379490
DP BARCODE:	359322 and 359309
TEST MATERIAL:	Test Substance TRYCRY1AB-0105, containing the truncated form of the full-length Cry1Ab protein
PROJECT STUDY NO:	SSB-165-08
SPONSOR:	Syngenta Seeds, Inc. - Field Crops - NAFTA
TESTING FACILITY:	Syngenta Biotechnology, Inc., Regulatory Science 3054 East Cornwallis Road, P.O. Box 12257 Research Triangle Park, NC 27709-2257.
TITLE OF REPORT:	Response to Data Deficiencies Noted for the Bt11 x MIR162 and Bt11 x MIR162 x MIR604 Applications for Registration [EPA Reg. No. 67979-RE and 67979-RG]
AUTHOR:	Huber S., Graser, G., and Ward, D.
STUDY COMPLETED:	November 19, 2008
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	Not GLP compliant- report represents a compilation of data from previously submitted studies. New data (figures) were generated according to accepted scientific methods and the raw data and records have been retained.
CONCLUSION:	The registrant submitted the mass spectra and a clearer reproduction of the Western blot gel as visual confirmation for establishing the molecular weight of the test substance TRYCRY1AB-0105, containing the *ca.* 66 kDa truncated form of the full length (*ca.* 130 kDa) Cry1Ab insecticidal protein. Total mass analysis of the Cry1Ab in test substance TRYCRY1AB found two predominant CrylAb species, with molecular weights of 66.3 and 65.8 kDa. Other supporting data include: insect bioassays demonstrating similar bioactivity of the test substance against ECB; N-terminal amino acid sequencing analysis of TRYCRY1AB-0105 in comparison to Cry1Ab protein expressed

in Event Bt11; and peptide mass analysis via Q-TOF comparing the masses of individual peptides resulting from proteolytic digestion of a test sample to the masses of known peptides in a database. In addition, field trial results comparing the efficacy of the single PIP events (Bt11, MIR162, and MIR604) to the combination PIP products: Bt11 x MIR162 and Bt11 x MIR162 x MIR604, showed no enhanced toxicity among various target pest species. Collectively, these data demonstrated the equivalence between the plant- and microbial-produced Cry1Ab proteins in support of utilizing the TRYCRY1AB-0105 test substance, as a suitable surrogate for the non-target organism toxicity studies submitted in support for the registration of the stacked Bt11 x MIR162 and Bt11 x MIR162 x MIR604 PIP products.

CLASSIFICATION: This report is rated as **ACCEPTABLE.** In addition, the classification of MRID No. 471372-11 is upgraded from supplemental to **ACCEPTABLE**, when combined with the results of this report.

I. STUDY SUMMARY

The purpose of this report is satisfy the data deficiencies noted in the characterization of TRYCRY1AB-0105, expressing Cry1Ab protein (MRID No. 471372-11), for use as a test substance surrogate for use in ecotoxicity studies submitted in support of the registration of the stacked Bt11 x MIR162 and Bt11 x MIR162 x MIR604 PIP products.

Test material:

Test substance TRYCRY1AB-0105 was prepared from archived cell paste of *E. coli* over-expressing the full-length Cry1Ab protein (The test substance for FLCry1Ab, FLCRY1AB-0103, contains 1181 amino acids and was originally characterized in Graser, 2005, MRID No. 470176-04). Bovine trypsin was used to partially hydrolyze the FLCry1Ab protein at two trypsin recognition sites: arginine-28 and arginine-619. The resulting, truncated protein was purified, pooled, and concentrated in 50mM NH_4HCO_3 (pH 9.25) and designated as trypsinized Cry1Ab or test substance TRYCRY1AB-0105.

The trypsinized Cry1Ab is similar to the truncated Cry1Ab expressed in Event Bt11 corn (see Figure 1), except that it lacks the first 28 amino acids, which are not required for insecticidal activity (Bietlot *et al.*, 1989). A schematic comparing the different versions of the Cry1Ab proteins is shown in Figure 1. The schematic shows: 1) FLCry1Ab protein, 2) the Cry1Ab protein produced in Event Bt11 corn, 3) the Event Bt11 Cry1Ab protein, treated with trypsin, and 4) the two Cry1Ab species contained in the TRYCRY1AB-0105 test substance.

II. RESULTS

A. N-Terminal Amino Acid Sequence Analysis

N-terminal amino acid sequence analysis was performed on the microbially produced Cry1Ab test substance, TRYCRY1AB-0105, and the Cry1Ab protein produced in Event Bt11 corn. As shown in Table 1, the sequence data from both analyses are consistent with the predicted N-terminal sequence.

TABLE 1. Comparison of N-terminal amino acid sequence data for bacterial- and plant-expressed Cry1Ab protein at position 29.

Cry1Ab protein Source	AA Sequence data
Event Bt11 corn [1]	?E ? ?Y ?PIDISL
TRYCRY1AB-0105 [2]	IE TGYTPIDISL
Predicted N-terminal sequence	IE TGYTPIDISL

[1] Meeusen and Mettler, 1994

[2] Kramer and Graser, 2007

B. Peptide Analysis

After proteolytic digestion of the TRYCRY1AB-0105 test sample, the resulting peptides were separated by high performance liquid chromatography (HPLC), measured by Quadrupole Time-of-Flight (Q-TOF2) spectrometer, and compared to the predicted Cry1Ab peptide sequences in a database. These peptide spectra were matched to the predicted sequence of trypsinized Cry1Ab, resulting in a total sequence coverage of 26% (see Figure 2, peptide matches are represented as bold and underlined).

B. Immunoreactivity

Western blot analysis of the TRYCRY1AB-0105 test substance revealed two immunoreactive bands (Figure 3, lanes 4 to 7) that cross-reacted to polyclonal antibodies to FLCry1Ab. These bands represented the *ca.* 66 kDa trypsinized Cry1Ab protein and the *ca.* 39 kDa Cry1Ab fragment derived from it. Corresponding bands in the Bt11 maize leaf extract were faint, but visible (see Figure 3, lane 2) and represented the intact Cry1Ab protein fragment at *ca.* 66.3 kDa, which had slightly slower mobility than the Cry1Ab protein from test substance TRYCRY1AB-0105. The difference in MW is consistent with the known 28 amino acid sequence added to the N-terminal end of the Cry1Ab protein in Event Bt11 maize (see Figure 1). The plant extract also contained additional breakdown products not observed in the test substance. However, the bands shown for the Bt11 plant extract (lane 2) are the same as those shown in the original Bt11 test substance equivalence report (Meeusen and Mettler 1994; MRID No. 43397502).

C. Molecular Weight Determination

Total mass analysis by HPLC/Q-TOF2 indicated that test substance TRYCRY1AB-0105 contained two major Cry1Ab protein species, both eluting with the same retention time of 13.6 minutes by HPLC (see Figure 4). The masses of these species were determined by Q-TOF2 to be 65.8 and 66.3 kDa and they were present in TRYCRY1AB-0105 in a ratio of approximately 2:1, respectively. The 66.3 kDa protein corresponded to the predicted

molecular weight of the trypsinized Cry1Ab protein as shown in Figure 1. The molecular weight of the 65.8 kDa fragment is consistent with a slightly shorter version of the Cry1Ab protein, missing the last four amino acids (DLER) at the C-terminus (Figure 1).

F. Insecticidal Activity

The bioactivity of TRYCRY1AB-0105 against first instar European corn borer (ECB) larvae showed a dose-response in insecticidal activity after oral exposure to the TRYCRY1AB-0105 test substance with a LC_{50} of 6.2 ng Cry1Ab /cm^2 (95 % confidence interval of 3.8 – 8.9 ng Cry1Ab /cm^2), with concentrations of ≥ 32.9 ng Cry1Ab /cm^2 causing 100 % lethality after 96 hours. These data confirm that the Cry1Ab present in test substance TRYCRY1AB-0105 was highly bioactive against ECB, a species known to be sensitive to Cry1Ab insecticidal protein.

G. Field studies on efficacy:

The efficacy of Bt11, MIR162, MIR604, Bt11 x MIR162, and Bt11 x MIR162 x MIR604 maize against several pests (including black cutworm, fall armyworm, European corn borer, and western corn rootworm) was assessed in field trials (Huber *et al.*, 2007 and White *et al.*, 2007a, b, c, and d). The results from a sub-set of the efficacy studies are presented in Figures 5-8. In all of the referenced studies, the efficacy of Bt11 x MIR162 and Bt11 x MIR162 x MIR604 maize was consistent with an additive effect of the individual efficacies of Bt11, MIR162, and MIR604 maize alone. The referenced efficacy studies corroborate the hypothesis that there is no interaction between the Cry1Ab, Vip3Aa20, and mCry3A proteins produced in the combined PIP products events Bt11 x MIR162, and Bt11 x MIR162 x MIR604 maize.

III. CONCLUSION

The registrant submitted the mass spectra and a clearer reproduction of the Western blot gel as visual confirmation for establishing the molecular weight of the test substance TRYCRY1AB-0105, containing the *ca.* 66 kDa truncated form of the full length (*ca.* 130 kDa) Cry1Ab insecticidal protein. These data demonstrated the equivalence between the plant- and microbial-produced Cry1Ab proteins in support of utilizing the TRYCRY1AB-0105 test substance, as a suitable surrogate for the non-target organism toxicity studies submitted in support for the registration of the stacked Bt11 x MIR162 and Bt11 x MIR162 x MIR604 PIP products. Therefore, the data deficiencies noted in the characterization of TRYCRY1AB-0105, expressing Cry1Ab protein (MRID No. 471372-11) have been satisfied.

IV. CLASSIFICATION

This report is rated as **ACCEPTABLE.** In addition, the classification of MRID No. 471372-11 is upgraded from supplemental to **ACCEPTABLE**, when combined with the results of this report.

V. REFERENCES

Bietlot H., Carey P.R., Choma C., Kaplan H., Lessard T., Pozsgay M. (1989) Facile preparation and characterization of the toxin from *Bacillus thuringiensis* var. *kurstaki*. *Biochem. J.* 260:87-91.

Graser G. (2005) *Characterization of Cry1Ab Test Substance FLCRY1AB-0103 and Certificate of Analysis*. Report No. SSB-001-05 (unpublished). Research Triangle Park, NC: Syngenta Biotechnology. US EPA MRID No. 47017604.

Huber S., White J., Mroczkiewicz S., and Ward D. (2007) *Insecticidal Efficacy Field Evaluations with MIR162 Maize Hybrids in 2005 and 2006.* Report No. SSB-522-07 (unpublished). Research Triangle Park, NC: Syngenta Biotechnology. US EPA MRID No. 47137816.

Kramer C. and Graser G. (2007) *Characterization of Trypsinized Cry1Ab Test Substance TRYCRY1AB-0105.* Report No. SSB-010-06 (unpublished). Research Triangle Park, NC: Syngenta Biotechnology. US EPA No. MRID No. 47137211.

Meeusen R. and Mettler I. (1994) *Equivalence of Plant and Microbially Produced Bacillus thuringiensis kurstaki* HD-1 Protein., Report No. NK5EQ (unpublished). Stanton, MN: Northup King Co. US EPA MRID No. 43397202.

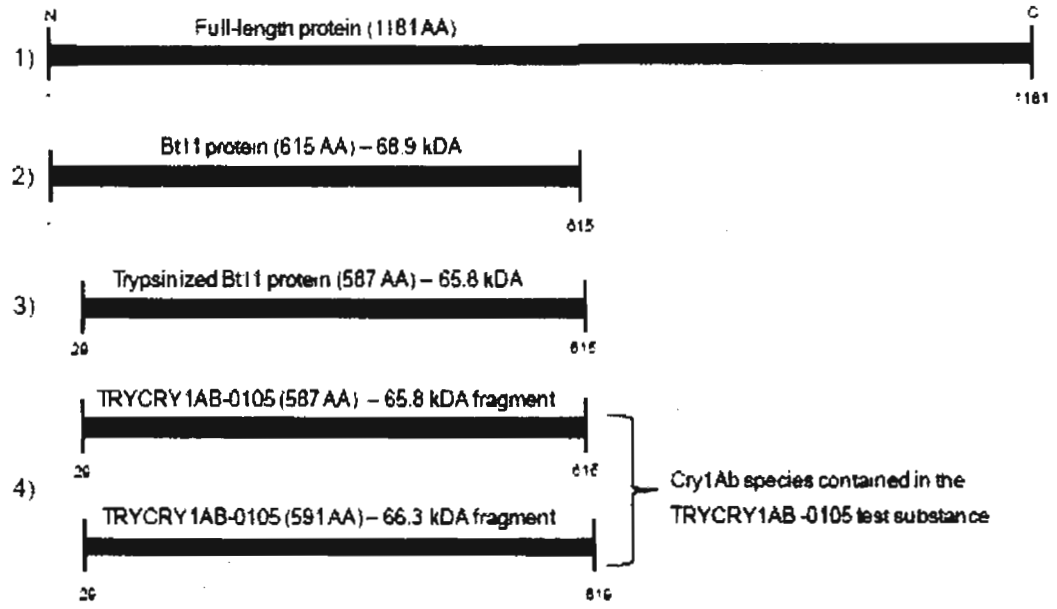
White J., Meehan M., and Meghji M. (2007a) *Insecticidal Efficacy of a Bt11 x MIR162 x MIR604 x GA21 Maize Hybrid against Corn Earworm in the Field.* Report No. SSB-510-07 (unpublished). Bloomington, IL: Syngenta Seeds. US EPA MRID No. 47153001.

White J., Sagers J., and Meghji M. (2007b) *Insecticidal Efficacy of a Bt11 x MIR162 x MIR604 x GA21 Maize Hybrid against European Corn Borer in the Field.* Report No. SSB-509-07(unpublished). Bloomington, IL: Syngenta Seeds. US EPA MRID No. 47153002.

White J., Sagers J., and Meghji M. (2007c)*Insecticidal Efficacy of a Bt11 x MIR162 x MIR604 x GA21 Maize Hybrid against Fall Armyworm in the Field.* Report No. SSB-511-07 (unpublished). Bloomington, IL: Syngenta Seeds. US EPA MRID No. 47153003.

White J., Sagers J., and Meghji M. (2007d) *Insecticidal Efficacy of a Bt11 x MIR162 x MIR604 x GA21 Maize Hybrid against Western Corn Rootworm in the Field.* Report No. SSB-515-07 (unpublished). Bloomington, IL: Syngenta Seeds. US EPA MRID No. 47153004.

Figure 1. Comparison of the different versions of Cry1Ab proteins
The fragments shown in orange represent the tryptic core of the full length Cry1Ab protein.



From MRID No. 476049-01, pg. 7, fig. 1

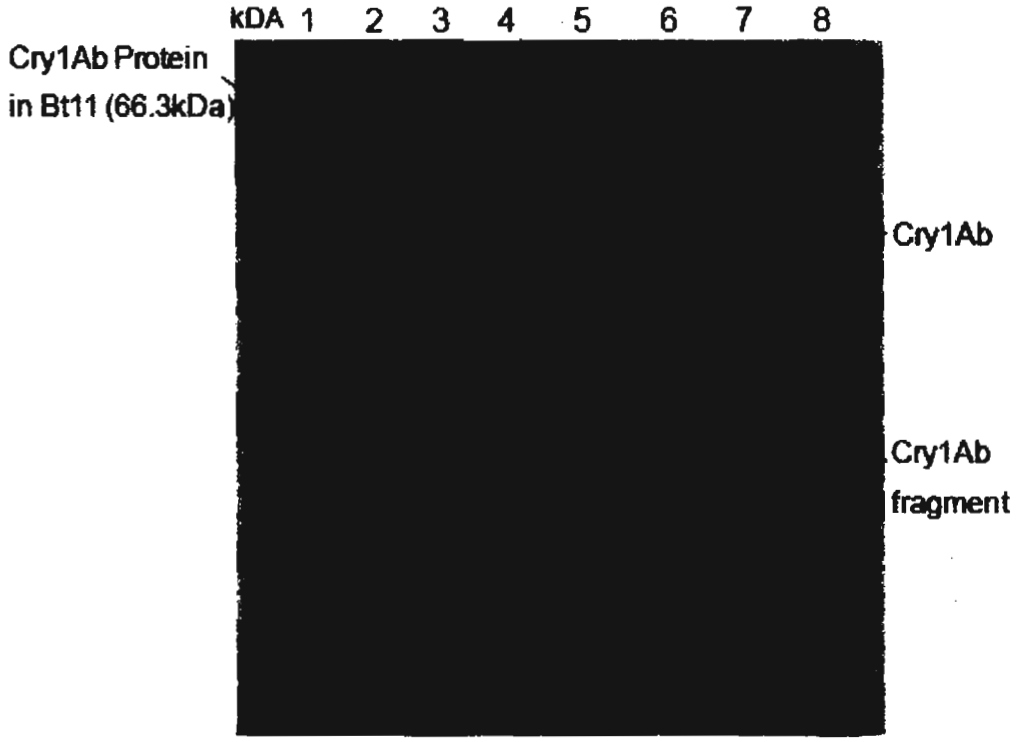
Figure 2. Peptide mapping of tryptic digest of TRYCRY1AB-0105 test substance.

IETGYTPIDISLSLTQFLLSEFVPGAGFVLGLVDIIWGIFGPSQWDAFLVQIEQLINQRIEE
FARNQAISRLEGLSNLYQIYAESFR**EWEADPTNPALREEMR**IQFNDMNSALTTAIPLFAVQN
YQVPLLSVYVQAANLHLSVLRDVSVFGQ**WGFDAATINSR**YNDLTRLIGNYTDHAVR**WYNTGL**
ERVWGPDSRDWIRYNQFR**RELTLTVLDIVSLFPNYDSR**TYPIRTVSQLTR**EIYTNPVLENFD**
GSFRGSAQGIEGSIRSPHLMDILNSITIYTDAHRGEYYWSGHQIMASPVGFSGPEFTFPLYG
TMGNAAPQQR**IVAQLGQGVYR**TLSSTLYRRPFNIGINNQQLSVLDGTEFAYGTSSNLPSAVY
R**KSGTVDSLDEIPPQNNNVPPR**QGFSHRLSHVSMFR**SGFSNSSVSIIR**APMFSWIHR**SAEFN**
NIIPSSQITQIPLTKSTNLGSGTSVVKGPGFTGGDILRRTSPGQISTLRVNITAPLSQRYRV
RIRYASTTNLQFHTSIDGRPINQGNFSATMSSGSNLQSGSFRTVGFTTPFNFSNGSSVFTLS
AHVFNSGNEVYIDRIEFVPAEVTFEAEY(DLER)

From MRID No. 476049-01, pg. 8, fig. 2

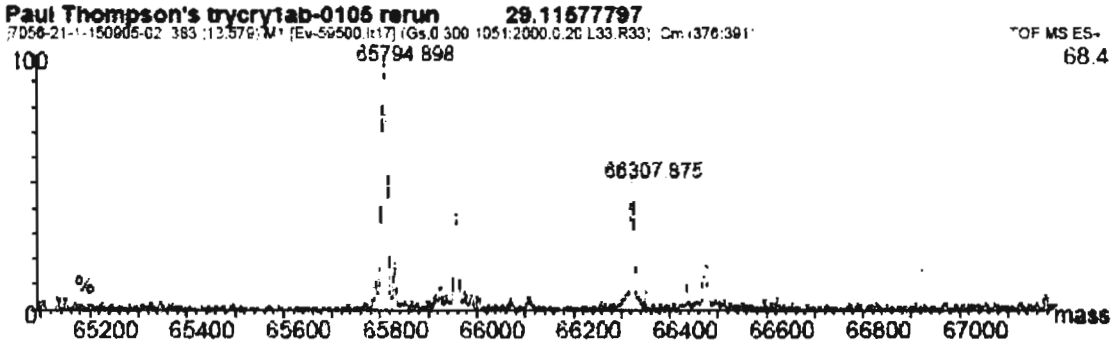
Figure 3. Immunoreactivity of Cry1Ab protein in test substance TRYCRY1AB-0105 and in Bt11 maize leave extracts (Western blot analysis) (Kramer and Graser 2007; MRID 47137211)

Lanes 1, 3 and 8: Molecular weight standard SeeBlue® Plus2 (Invitrogen; CA, USA)
Lanes 2: Event Bt11 maize leaf extract (20 ng)
Lanes 4 to 7: *ca.* 38, 29, 19, and 5 ng Cry1Ab, respectively, from TRYCRY1AB-0105



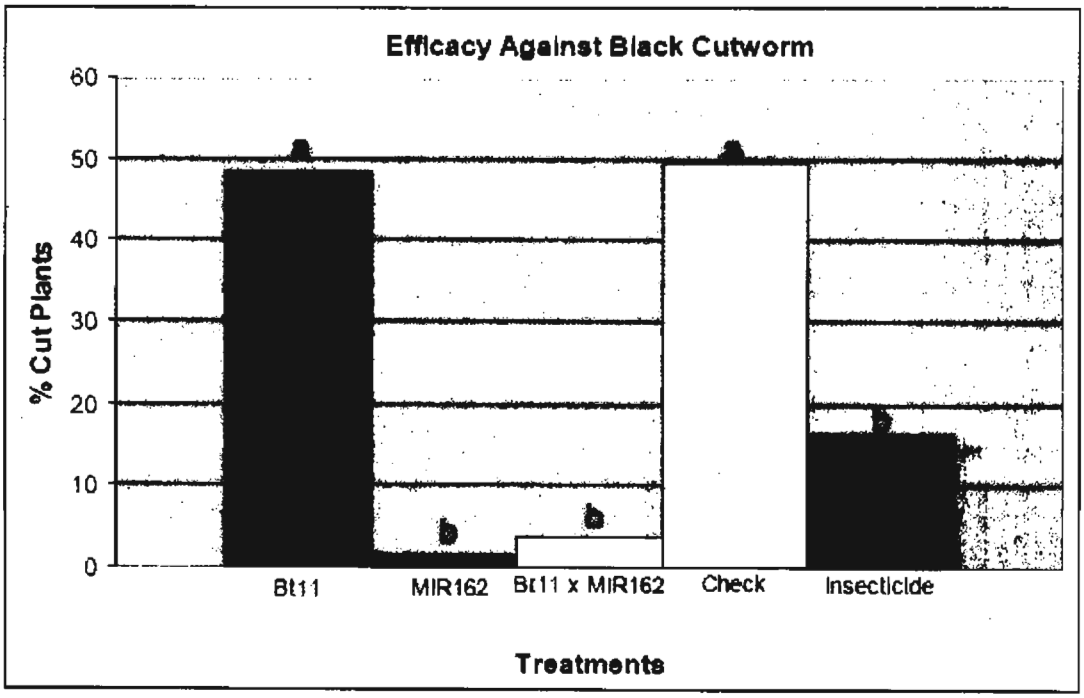
From MRID No. 476049-01, pg. 9, fig. 3

Figure 4. Deconvoluted mass spectrum TRYCRY1AB-0105 test substance of the peaks eluting at 13.6 minutes.



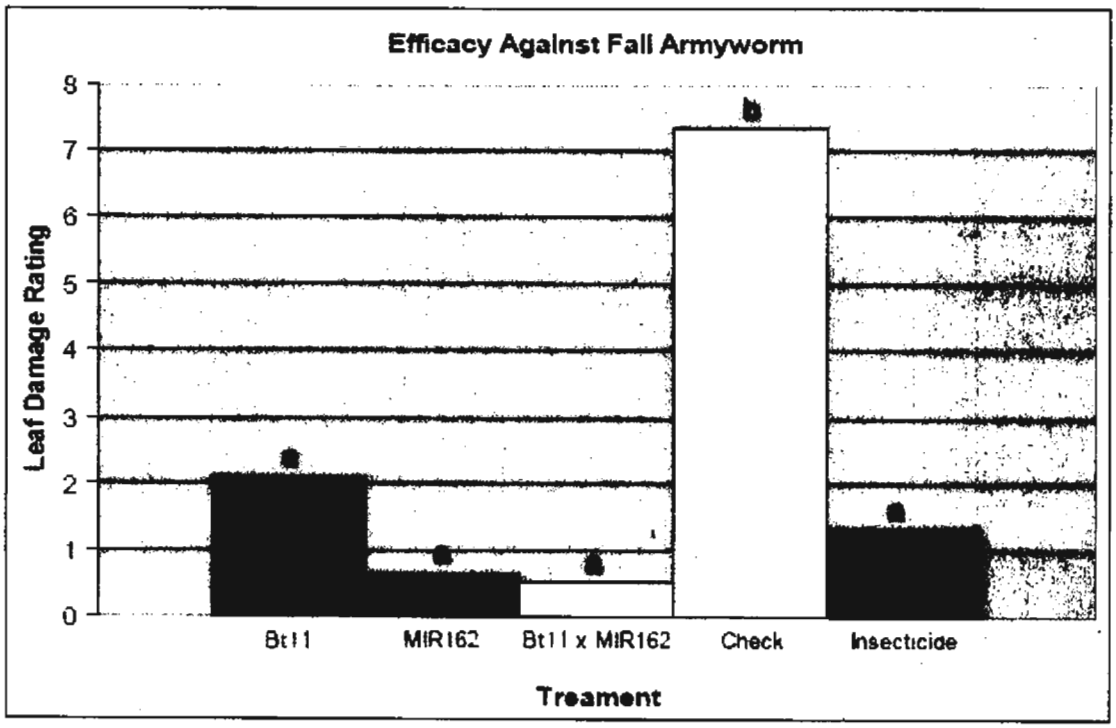
From MRID No. 476049-01, pg. 10, fig. 4

Figure 5. Efficacy against black cutworm (Huber *et al.*, 2007; MRID 471378816)
Summary results of year 2005 black cutworm efficacy trial results with maize hybrids containing events Bt11, MIR162 and Bt11 x MIR162. The percent of cut plants in each plot was determined 14 days post-infestation and combined-location means were computed for each entry. The total number of plots rated equaled 16 per entry. A combined-location ANOVA was performed and the LSD value at $p<0.05$ was determined to be 17.5. Significant differences between entry means are denoted by letter code: 'b' is significantly different from 'a'.



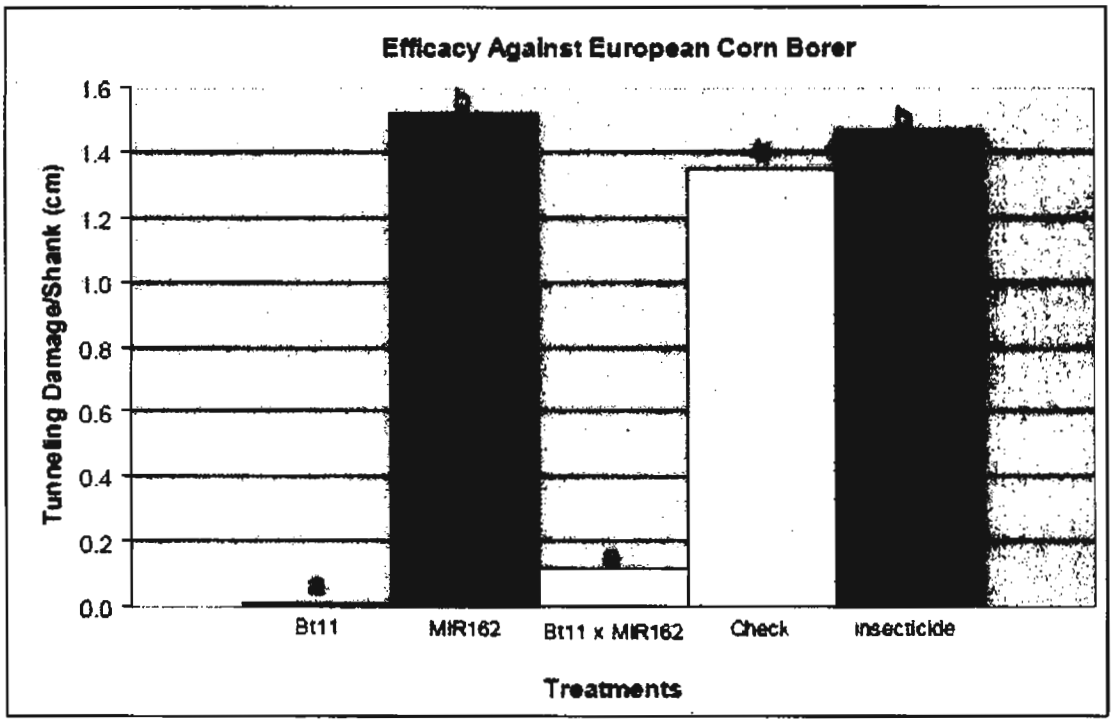
From MRID No. 476049-01, pg. 14, fig. 5

Figure 6. Efficacy against fall armyworm (Huber *et al.*, 2007; MRID 471378816)
Summary results of year 2005 fall armyworm efficacy trial results with maize hybrids containing events Bt11, MIR162 and Bt11 x MIR162. The mean leaf damage rating in each plot was determined 14 days post-infestation and combined-location means were computed for each entry. The total number of plots rated equaled 8 per entry. A combined-location ANOVA was performed and the LSD value at $p<0.05$ was determined to be 3.9. Significant differences between entry means are denoted by letter code: 'b' is significantly different from 'a'.



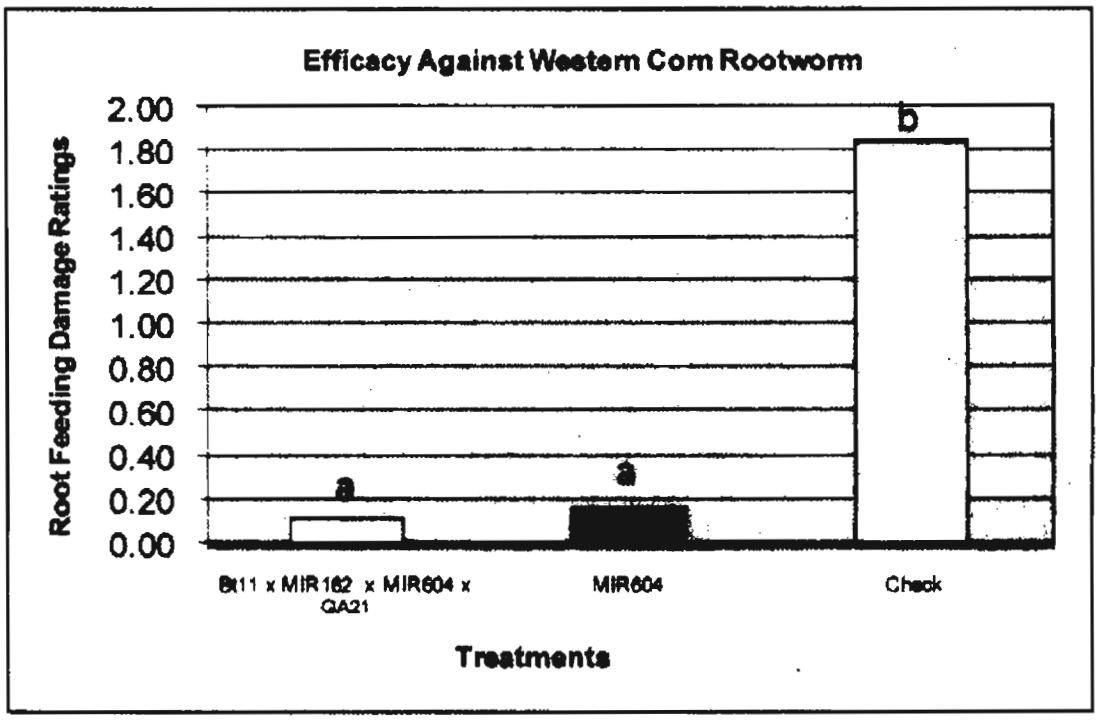
From MRID No. 476049-01, pg . 15, fig. 6

Figure 7. Efficacy against European corn borer (Huber *et al.*, 2007; MRID 471378816) Summary results of year 2005 European corn borer trial results with maize hybrids containing events Bt11, MIR162 and Bt11 x MIR162. The mean tunnel length in each plot was determined at physiological maturity (R6) and combined-location means were computed for each entry. The total number of plots rated equaled 16 per entry. A combined-location ANOVA was performed and the LSD value at $p<0.05$ was determined to be 1.2. Significant differences between entry means are denoted by letter code: 'b' is significantly different from 'a'.



From MRID No. 476049-01, pg. 16, fig. 7

Figure 8. Efficacy against western corn rootworm (White *et al.*, 2007d; MRID 47153004) Summary results of year 2007 Western Corn Rootworm trial results with maize hybrids containing events MIR604 and Bt 11 x MIR162 x MIR604. The mean root feeding damage rating in each plot was determined at silking (R1 stage of plant development) and combined location means were computed for each entry. The total number of plots rated equaled 8 per entry. A combined-location ANOVA was performed and the LSD value at $p<0.05$ was determined to be 0.23. Significant differences between entry means are denoted by letter code: 'b' is significantly different from 'a'.



From MRID No. 476049-01, pg. 17, fig. 8