13) Li, M. H. and E. H. Robinson (1999). Evaluation of insect protected corn lines MON 853 and MON 859 as a feed ingredient for catfish. MSL-16164, an unpublished study conducted by Monsanto Company.

FINAL REPORT

Evaluation of Insect Protected Corn Lines MON 853 and MON 859 as a Feed Ingredient for Catfish

Monsanto Study Number: XX-98-297

Monsanto Report Number: MSL 16164

Submitted to: Monsanto Company (Sponsor)
700 Chesterfield Parkway, North
Chesterfield, MO 63198

Study Initiation Date: December 02, 1998

Study Completion Date: August 09, 1999 (Amended)

Authors: Meng H. Li and Edwin H. Robinson

Thad Cochran National Warmwater Aquaculture Center (Testing Facility)
Mississippi State University
127 Experiment Station Road
P.O. Box 197
Stoneville, MS 38776-0197

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d)(l)(A), (B), or (C).

"We submit this material to the United States Environmental Protection Agency specifically under provisions contained in FIFRA as amended, and thereby consent to use and disclosure of this material by EPA according to FIFRA. In submitting this material to the EPA according to method and format requirements contained in PR Notice 86-5, we do not waive any protection of rights involving this material that would have been claimed by the company if this material had not been submitted to the EPA."

Company: Monsanto Company

Company Agent:

William P. Pilacinski
Regulatory Affairs Manager

Date

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT (AMENDED)

This study was conducted in compliance with the United States EPA FIFRA Good Laboratory Practice Regulations (40 CFR Part 160).

Study Director: 08-09-99

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Date

QUALITY ASSURANCE UNIT STATEMENT

This study was examined for conformance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160, 17 August 1989. Reviews conducted by another Quality Assurance Unit are included in the Quality Assurance Statement for the analytical summary in Appendix B.

Dates of Inspection/Audit	Phase	Date Reported to Study Director	Date Reported to Management
12/09/98	Preparation of Catfish Diet	12/21/98	12/21/98
01/13/99	Counting and Weighing Catfish	01/21/99	01/21/99
03/18/99	Review of Raw Data Notebook	03/22/99	03/22/99
06/25/99	Review of Final Report	07/01/99	07/01/99

Gene D Wills

07/21/99

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Date

REPORT CERTIFICATION (AMENDED)

This final report is a true and accurate account of the work conducted in the study.

Certified by:

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08-09-99
Date

TABLE OF CONTENTS (AMENDED)

SUMMARY (AMENDED)

Study Title: Evaluation of Insect Protected Corn Lines MON 853 and MON 859 as a Feed Ingredient for Catfish

Sponsor: Monsanto Company

Testing Facility: Thad Cochran National Warmwater Aquaculture Center (NWAC)
Mississippi State University

Study Initiation Date: December 02, 1998

Test Dates: Acclimation - December 03 to 15, 1998
Fish Stocking - December 16, 1998
Feeding - December 17, 1998 to February 10, 1999
Final Weighing and Sampling - February 11, 1999
Sample Analyses - February 12 to 26, 1999

Study Termination Date: August 09, 1999 (Amended)

Level of Test Substances: 35% in the diet

Test System: Channel catfish (*Ictalurus punctatus*)

Age of Test System: 6 months of age

Source of Test System: NWAC, Mississippi State University

Results:

There were no significant differences in feed consumption, weight gain, feed conversion ratio, survival, percentage visceral fat, or percentages fat, protein, or ash in fillets of channel catfish fed diets containing insect protected corn grain or non-genetically modified corn grain. No abnormal fish behavior was noticed during the study. Significant differences were observed in percentage fillet moisture among fish fed different diets. Fish fed the diet containing corn grain of the line MON 859 had a lower fillet moisture than fish fed the diet containing corn grain of the line MON 846 which was the non-genetically modified parent line of MON 859, while fillet moisture of fish fed the diet containing corn grain of the line MON 853 was not different from those fed the diet containing corn grain of the line MON 847, the non-genetically modified parent line of MON 853. Fish fed diets containing genetically modified corn grain (MON 853 and MON 859) had a percentage fillet moisture similar to fish fed the diet containing corn grain of a commercial hybrid (H 2390). The apparent statistical differences in fillet moisture are relatively unremarkable and unlikely diet related.

INTRODUCTION

Corn has been genetically modified to express the Cry3Bb1 protein from *Bacillus thuringiensis* which has insecticidal activity against certain insect pests. The objective of this study was to assess the growth and survival of channel catfish fed diets containing corn grain derived from genetically modified insect protected corn lines as compared to channel catfish fed diets containing parental control corn lines and a commercial hybrid.

MATERIALS AND METHODS

Test, Control, and Reference Substances

The test substances were finely ground corn grain of two insect protected corn lines MON 853 and MON 859 prepared by the sponsor. The lot numbers of the test substances were MON 85328 and MON 85928, respectively. These corn lines have been genetically modified to contain the Cry3Bb1 protein. Corn lines MON 853 and MON 859 were selected for this study because they had the highest level of Cry3Bb1 protein of several corn lines being developed (Monsanto Study Plan 98-01-39-08). The Cry3Bb1 protein was previously referred to as CryIIIB2 (or Cry3B2) as well as Cry3Bb or CryIIIC. According to the most recent and accepted nomenclature, this protein should be referred to as Cry3Bb1 (Crickmore *et al.* 1998). The Cry3Bb1 nomenclature will be used in this report.

The control substances were finely ground corn grain of two non-genetically modified corn lines with similar genetics to the test substance prepared by the sponsor. MON 846 was the control for corn line MON 859 and MON 847 was the control for corn line MON 853. The lot numbers of the control substances were MON 84628 and MON 84728, respectively.

The reference substance was finely ground corn grain from a commercial corn hybrid 2390 prepared by the sponsor. The reference substance was included to assess how commercial corn hybrids perform in this assay.

The test, control, and reference substances were received on December 04, 1998.

Diet Preparation

The test, control, and reference substance diets were based on typical channel catfish feeds (Table 1) and formulated to contain approximately 32% crude protein using ground corn, soybean meal, menhaden fish meal, meat and bone/blood meal, and wheat middlings as the primary ingredients. Vitamin and mineral supplements were added in each diet. Each diet consisted of 35% ground corn. The diets were formulated using MIXIT-2 Version 3.3 computer software of Agricultural Consultants, Inc. (Kingsville, TX). All known nutrient requirements of channel catfish were met (NRC 1993).

The diets were prepared at the Thad Cochran National Warmwater Aquaculture Center (NWAC)

according to the NWAC Catfish Nutrition Laboratory Standard Operating Procedure SOP005-98-1, which was based on procedures described by Li *et al.* (1993). A 4-kg batch was made for each diet. Dietary ingredients were weighed using a calibrated electronic balance and mixed in a V-mixer for 20 minutes, then placed in a dough mixer where catfish oil (1.5%) and distilled water (30%) were added and mixed for 10 minutes, respectively. The feed mixture was passed through a die in a meat grinder. The resulting feed strands were dried in a forced air oven at a temperature of 29 to 33°C for 2.5 to 3 hours. The dried feed was broken into small particles by means of a food blender, screened to remove feed dust, mixed, and stored in sealed plastic bags at approximately - 20°C until fed.

Test System

The test system was defined as channel catfish, *Ictalurus punctatus*. The Kansas strain of channel catfish was used. Fish were raised from eggs on site at the NWAC. Channel catfish were justified as the test system because corn grain is used as a feed ingredient in commercial channel catfish feeds. Each aquarium (containing 20 channel catfish) was identified by a waterproof label with the study number, diet number, and aquarium number.

Fish Culture

Channel catfish were hatched on site at the NWAC in May 1998 and raised indoors to an average weight of approximately 2.8 g/fish at which time they were moved into a holding tank located in NWAC Catfish Nutrition Laboratory. During the holding period they were fed a commercial catfish feed (36% protein floating fingerling feed) at approximately 3% body weight daily.

Experimental Design

Two weeks before initiation of the study, 30 fish were transferred into each of 25 aquaria located inside the wet laboratory at the NWAC for acclimation. These aquaria were part of a flowing water system in the wet laboratory. Local well water was used as the water source for the study. Each aquarium was supplied with well water at a flow rate of approximately 1 liter per minute and continuous aeration. During this period the fish were fed a 32% protein diet twice daily to approximate satiation.

After acclimation, all fish were pooled into one aquarium and graded to a uniform size. Twenty fish of similar size were restocked into each of the 25 aquaria. The average initial weight of fish was 4.6 g/fish. Five randomized aquaria were used for each of the five dietary treatments. The diet of each corn line was fed to 100 catfish distributed evenly among five 80-liter (water volume) glass aquaria. A total of 500 fish were used in the study. A one-way analysis of variance was conducted on total weight of fish by aquarium to assure that no significant variation in fish weight was present at initiation of the study.

All fish were fed twice daily (0800 to 0900 and 1600 to 1700 hours) to approximate satiation for 8 weeks based on percentage of fish body weight. The fish were allowed to eat for 20 minutes

after which feeding activity was recorded.

Observations

Fish in each aquarium were counted and weighed collectively at the initiation and weeks 4 and 8 of the feeding period to determine total number and weight of fish in each tank. Mortality and behavior were observed and recorded daily. Water temperature and dissolved oxygen were monitored daily using an oxygen/temperature meter. Target water temperature was 30 ± 2 °C and dissolved oxygen at 5 mg/liter and above.

Analyses of Diet Samples

Prior to initiation of the study, a 10-g sample of freshly prepared diets was collected and ground through a # 40 mesh screen using a sample grinder. Proximate analyses were performed on all diets prior to initiation of the study according to NWAC Catfish Nutrition Laboratory Standard Operating Procedures SOP001-98-1, SOP002-98-1, SOP003-98-1, and SOP004-98-1, which were based on standard AOAC (Association of Official Analytical Chemists International) methods (AOAC 1995). Proximate analyses refer to the determination of dry matter/moisture, crude protein, crude fat, and ash.

Dry matter was determined by oven drying at 135 °C for 2 hours. Ash was determined by wet ashing on a hot plate using nitric acid and then dry ashing by muffle furnace at 600 °C for 12-16 hours. Crude protein was determined by automated Kjeldahl method. Samples were digested with sulfuric acid and hydrogen peroxide on a digestion block under a fume hood for 1 hour. Then sodium hydroxide solution was added to release ammonia which was distilled by steam and titrated with standard hydrochloric acid solution. Crude fat was determined using ether extraction method. Samples were extracted for fat by ether for 45 minutes and fat in each sample was collected into an aluminum cup which was dried in an oven at 100-102 °C for 30 minutes before and after the extraction process.

In addition to the proximate analyses, diet samples were collected at initiation and termination of the study and returned to the sponsor on dry ice. These samples were used to confirm the presence of the Cry3Bb1 protein. The samples were analyzed using ELISA in accordance with Monsanto SOP # BRME-0059-03.

Analyses of Fish Fillet Samples

At the end of the feeding period, 5 fish from each aquarium (25 fish per treatment) were sacrificed by overdose with tricaine methanesulfonate (MS-222) and fillets taken and pooled by aquarium for subsequent proximate analyses. Proximate analyses were performed on fish fillet samples according to NWAC Catfish Nutrition Laboratory Standard Operating Procedures SOP001-98-1, SOP002-98-1, SOP003-98-1, and SOP004-98-1.

Approximately a 40-g fillet sample from each tank was ground into paste. Approximately a 25-g

ground fillet sample from each tank was freeze-dried for a minimum of 12 hours. The freeze-dried samples were used to determine crude protein and crude fat. The remaining wet samples were used to determine moisture and ash. Procedures for proximate analyses of fillet samples were the same as described for diet samples except that fillet moisture was determined by oven drying at 100-102 °C for 16-18 hours.

Data Collection

Data collected during this study included:

1. Feed consumption (total feed consumed/number of fish, g/fish)
2. Weight gain (final weight of fish - initial weight of fish, g/fish)
3. Feed conversion ratio (feed consumption/weight gain)
4. Visceral fat (visceral fat/whole fish weight, %)
5. Proximate composition of diets and fish fillets (% moisture, % crude protein, % crude fat, % ash)
6. Daily observations for adverse effects including mortality and behavioral changes.

Data Calculations

Raw data collected were entered to the Microsoft Excel 97 spreadsheet and calculated to obtain data list in **Data Collection**. Mean and standard deviation of dry matter, crude protein, crude fat, and ash concentration of diet samples were calculated. Feed consumption, weight gain, feed conversion ratio, survival, visceral fat, and fillet moisture, protein, fat, and ash from each tank were calculated and subsequently analyzed statistically.

Statistical Analysis

A one-way analysis of variance (ANOVA) was conducted on initial weight of fish, feed consumption, weight gain, feed conversion ratio, percentage visceral fat, and percentages moisture, ash, protein, and fat of fish fillets (Steel and Torrie 1980) using the Statistical Analysis System (SAS) for Windows, Release 6.12 (The SAS Institute, Cary, North Carolina). Least significant difference (LSD) test was conducted only if ANOVA indicated $p \leq 0.05$. Fisher's exact test of equal proportions was performed on mortality/survival data using SAS.

Storage Locations of Specimens, Raw Data, QAU Report, and Final Report

The original final report, the original supporting raw data, and the original facility records will be submitted to the Study Sponsor for archiving. A certified copy of the final report, a certified copy of the supporting raw data, and a certified copy of the facility records will be archived at the NWAC. Remaining test materials and diets were returned to the Sponsor. Remaining fish tissue samples are stored at approximately - 20°C in Freezer # 6 in the NWAC Catfish Nutrition Laboratory. QUA reports are stored in a fire-proof cabinet in the Study Director's office.

RESULTS AND DISCUSSION (AMENDED)

Proximate analyses were performed on all diets prior to initiation of the study. Mean and standard deviation of dry matter, crude protein, crude fat, and ash concentrations of diet samples were calculated (Table 2). No notable difference among the diets for the different corn lines was observed. In addition, the Cry3Bb1 protein was confirmed to be present in the test substance diets and absent in the control and reference substance diets (Appendix B).

Survival and growth of fish exposed to the test, control, and reference substances are shown in Table 3. Feed consumption, weight gain, feed conversion ratio, and survival of channel catfish fed diets containing insect protected corn grain were not different from those fed diets containing non-genetically modified corn grain or a diet containing commercial corn grain after eight weeks of feeding (Table 4). Survival of fish ranged from 99% to 100% in all dietary treatments. No abnormal fish behavior was noticed in any tank. No differences in percentage visceral fat or percentages fillet fat, protein, or ash were found among channel catfish fed the different experimental diets (Table 5).

Significant differences were observed in percentage fillet moisture among fish fed different diets (Table 5). Fish fed the diet containing corn grain of the line MON 859 had a lower fillet moisture than fish fed the diet containing corn grain of the line MON 846 which was the non-genetically modified parent line of MON 859, while fillet moisture of fish fed the diet containing corn grain of the line MON 853 was not different from those fed the diet containing corn grain of the MON 847, the non-genetically modified parent line of MON 853. Fish fed diets containing genetically modified corn grain (MON 853 and MON 859) had a percentage fillet moisture similar to fish fed the diet containing corn grain of a commercial hybrid H 2390. The apparent statistical differences in fillet moisture are relatively unremarkable and unlikely diet related.

CONCLUSION

Results from this study indicate that corn grain derived from the two insect protected corn lines, MON 853 and MON 859 can be used as a feed ingredient in channel catfish diet at levels of up to 35% without adverse effect on fish growth, feed conversion efficiency, survival, behavior, or body composition.

REFERENCES

AOAC (Association of Official Analytical Chemists International). 1995. Official methods of analysis, 16th edition. Arlington, Virginia.

Crickmore, N., D. R. Zeigler, J. Feitelson, E. Schnepf, J. Van Rie, D. Lereclus, J. Baum, and D. H. Dean. 1998. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. Microbiol Mol Biol Rev 62:807-13.

Li, M. H., M. R. Johnson and E. H. Robinson. 1993. Elevated dietary vitamin C concentrations did not increase resistance of channel catfish, *Ictalurus punctatus* against *Edwardsiella ictaluri* infection. Aquaculture 117:303-312.

NRC (National Research Council). 1993. Nutritional requirements of fish. National Academy Press, Washington, D.C.

Robinson, E. H. and M. H. Li. 1996. A practical guide to nutrition, feeds, and feeding of catfish. Bulletin 1041, Mississippi Agricultural and Forestry Experiment Station, Mississippi State, Mississippi.

Steel, R. G. and J. H. Torrie. 1980. Principles and procedures of statistics, a biometric approach, 2nd edition, McGraw-Hill, New York, New York.

Table 1. Ingredient composition of experimental diets (expressed on an as-fed basis).

Ingredient	%
Corn meal	35.0
Soybean meal	39.7
Cottonseed meal	10.0
Menhaden fish meal	4.0
Meat, bone, blood meal	4.0
Wheat middlings	2.6
Dicalcium phosphate	1.0
C-free vitamin premix[1]	0.1
Trace mineral premix[1]	0.1
Vitamin C[2]	0.06
Carboxymethyl cellulose[3]	2.0
Catfish oil	1.5

[1] Catfish vitamin and trace mineral premixes as described by Robinson and Li (1996).
[2] Stay C™ (25% active, Hoffmann LaRoche, Inc., Nutley, NJ).
[3] Pellet binder.

Table 2. Dry matter, crude protein, crude fat, and ash concentrations of experimental diets (as-fed basis). Values represent means of duplicate analyses (standard deviation).

Diet number	Corn line number	Dry matter (%)	Crude protein (%)	Crude fat (%)	Ash (%)
1	H 2390	85.80 (0.01)	30.76 (0.17)	3.76 (0.14)	6.10 (0.10)
2	MON846	88.52 (0.01)	32.17 (0.45)	3.74 (0.02)	6.39 (0.13)
3	MON847	86.73 (0.04)	32.16 (0.01)	3.85 (0.01)	6.18 (0.11)
4	MON853	86.25 (0.04)	31.51 (0.24)	3.92 (0.01)	6.13 (0.11)
5	MON859	87.33 (0.08)	32.37 (0.11)	3.80 (0.03)	6.38 (0.08)

Table 3. Fish number and weight in each aquarium, and average weight per fish at the initiation, week 4, and week 8 (at the end) of the feeding period.

Diet number	Corn line number	Aquarium number	At the initiation			At week 4			At week 8		
			Number	Weight (g/aquarium)	Weight (g/fish)	Number	Weight (g/aquarium)	Weight (g/fish)	Number	Weight (g/aquarium)	Weight (g/fish)
1	H 2390	6	20	94.9	4.7	19	262.5	13.8	19	663.2	34.9
1	H 2390	8	20	92.9	4.6	20	255.3	12.8	20	615.9	30.8
1	H 2390	12	20	92.0	4.6	20	256.0	12.8	20	640.3	32.0
1	H 2390	13	20	92.7	4.6	20	251.7	12.6	20	622.0	31.1
1	H 2390	19	20	91.2	4.6	20	256.3	12.8	20	646.2	32.3
2	MON 846	7	20	92.8	4.6	20	261.1	13.1	20	643.8	32.2
2	MON 846	15	20	92.8	4.6	20	256.7	12.8	20	643.5	32.2
2	MON 846	16	20	92.3	4.6	20	253.9	12.7	20	634.2	31.7
2	MON 846	21	20	91.0	4.6	20	256.2	12.8	20	635.4	31.8
2	MON 846	25	20	93.3	4.7	20	260.3	13.0	20	630.8	31.5
3	MON 847	1	20	92.6	4.6	20	254.4	12.7	20	646.9	32.3
3	MON 847	14	20	92.9	4.6	20	261.7	13.1	20	643.8	32.2
3	MON 847	18	20	93.3	4.7	20	257.8	12.9	20	651.8	32.6
3	MON 847	20	20	90.3	4.5	20	256.5	12.8	20	635.9	31.8
3	MON 847	23	20	94.4	4.7	20	269.1	13.5	20	664.2	33.2
4	MON 853	3	20	93.0	4.7	20	262.6	13.1	20	667.0	33.4
4	MON 853	5	20	91.2	4.6	20	259.8	13.0	20	650.4	32.5
4	MON 853	10	20	91.1	4.6	20	259.3	13.0	20	647.6	32.4
4	MON 853	11	20	93.4	4.7	20	260.8	13.0	20	645.8	32.3
4	MON 853	22	20	92.4	4.6	20	255.6	12.8	20	634.2	31.7

Continued

Table 3, continued

5	MON 859	2	20	92.9	4.6	20	256.1	12.8	20	639.9	32.0
5	MON 859	4	20	90.7	4.5	20	251.3	12.6	19	626.7	33.0
5	MON 859	9	20	92.6	4.6	20	253.5	12.7	20	622.6	31.1
5	MON 859	17	20	91.4	4.6	20	254.6	12.7	20	621.2	31.1
5	MON 859	24	20	92.4	4.6	20	252.8	12.6	20	666.2	33.3

Table 4. Mean feed consumption, weight gain, feed conversion ratio, and survival of channel catfish fed experimental diets. Means in each column were found not to differ ($p > 0.05$, one-way analysis of variance).

Diet number	Corn line number	Feed consumption (g/fish)	Weight gain[1] (g/fish)	Feed conversion ratio[2]	Survival (%)
1	H 2390	32.9	27.6	1.19	99.0
2	MON 846	32.6	27.3	1.20	100.0
3	MON 847	32.7	27.8	1.18	100.0
4	MON 853	32.7	27.8	1.17	100.0
5	MON 859	32.2	27.5	1.17	99.0

[1]Mean initial weight was 4.6 g/fish.

[2]Feed conversion ratio = weight of feed consumption (based on 90% dry matter)/weight gain of live fish.

Table 5. Mean visceral fat, fillet moisture, fat, protein. and ash (on a wet tissue basis) of channel catfish fed experimental diets. Means in each column were found not to differ ($p > 0.05$) except for means of fillet moisture which were different ($p \leq 0.05$, one way analysis of variance). Means of fillet moisture followed by different letters were different ($p \leq 0.05$, least significant difference test).

Diet number	Corn line number	Visceral fat (%)	Fillet moisture (%)	Fillet fat (%)	Fillet protein (%)	Fillet ash (%)
1	H2390	1.80	78.4 bc	2.19	18.3	1.15
2	MON846	1.50	78.8 a	1.94	17.5	1.07
3	MON847	1.83	78.7 ab	2.16	17.8	1.17
4	MON853	1.54	78.4 bc	2.30	18.3	1.13
5	MON859	1.73	78.2 c	2.27	18.1	1.13
LSD[1]			0.38			

[1]Least significant difference.

APPENDIX A

PERSONNEL INVOLVED IN THIS STUDY

The following personnel were involved in the conduct of this study:

Tom Campbell, Student Worker
Carol Dennis, Fishery Aide
Margaret Dennis, Research Assistant I
Meng Li, Assistant Fishery Biologist
Amy Morris, Fishery Aide
Danny Oberle, Research Assistant II
Sandra Phillipps, Fishery Technician
Cliff Smith, Research Technician

APPENDIX B

DOSE ANALYSIS

Quality Assurance Unit Statement for Analytical Subreport

Study Title: Evaluation of Insect Protected Corn Lines MON 853 and MON 859 as a Feed Ingredient for Catfish

Reviews conducted by the QAU confirm that the final analytical summary reflects the raw data.

Following is a list of reviews conducted by the Monsanto AG Regulatory QAU on the study reported herein.

Dates Of Inspection / Audit	Phase	Date Reported To: Study Director	Management
June15, 1999	Analytical sub-Report and Data Audit	June 23, 1999	June16, 1999
July 9, 1999	Final Review	July12, 1999	July12, 1999

James L. Ogilvie
Quality Assurance Specialist
AG Regulatory, Monsanto Company

Date July 20, 1999

Activity: Qualitative ELISA Analysis of Catfish Diet from Study # XX-98-297

Principal Investigator: Yelena A. Dudin

Performing Laboratory: Monsanto Company
Biotechnology Regulatory Sciences
700 Chesterfield Parkway North
St. Louis, MO 63198

Objective:

The objective of this analysis was to evaluate each of the diets used in the catfish study to confirm the presence or absence of the *Bacillus thuringiensis* (*B.t.*) protein in the test, control and reference substances.

Samples analyzed:

A study to assess the potential effects on catfish diets prepared with grain from genetically modified corn was conducted at Mississippi State University. The corn had been genetically modified to express *B.t.* protein for protection against certain insect pests. Samples of the catfish diet were collected at initiation and termination of the study and returned to Monsanto for analysis using qualitative ELISA (enzyme linked immunosorbent assay).

Methods:

The fish diet was extracted and measured as described in the study note to file.

Results:

Diets prepared from grain of MON 846, MON 847 and H2390 did not contain the *B.t.* protein, confirming their identity as control and reference diets. The *B.t.* protein was confirmed to be present in the samples of catfish diets prepared from MON 853 and MON 859 corn grain. The raw data for this analysis is located in the Monsanto Study File XX-98-297.

Conclusions:

Catfish diets prepared using grain from MON 853 and MON 859 were found to contain the *B.t.* protein of interest. The non-transgenic corn lines (MON 846, MON 847 and commercial line H2390) did not contain the *B.t.* protein. These data serve to confirm the phenotype of the test, control and reference substances as evidenced by the presence or absence of the *B.t.* protein.

Summary Prepared By: _____ Date: 7/20/99

APPENDIX C

REPORT AMENDMENT # 1

We corrected the reference substance number on Page 7, Line 30 and Page 12, Line 22 from H 2349 to H 2390, which was a typographical error. There was no impact on the integrity of the study. This amendment has been reviewed by Gene D. Wills, Quality Assurance Representative.

Study Director:

Meng Li — Date: 08-09-99

Sponsor Representative:

Mike McKee — Date: 8/11/99