TOXICOLOGY REPORT SUMMARY

Summary of Study CV-2000-260: 13-Week Dietary Subchronic Comparison Study with MON 863 Corn in Rats Preceded by a 1-Week Baseline Food Consumption Determination with PMI Certified Rodent Diet #5002

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ABSTRACT

Monsanto Company has developed corn event MON 863, which protects corn plants against feeding damage from corn rootworm larvae (*Diabrotica* spp.), a major North American insect pest. Event MON 863 contains a coding sequence that expresses a variant of the wild-type Cry3Bb1 insecticidal protein from *Bacillus thuringiensis*. This study included the test event MON 863, and the nontransgenic control line, LH82 x A634, which has background genetics representative of the test event but does not contain the *cry3Bb1* insect control coding sequence.

This study was undertaken to compare the responses of rats fed diets containing grain derived from MON 863 corn to rats fed diets containing grain from the nontransgenic control line and a population of rats fed reference control diets containing grain from six different commercially available nontransgenic corn varieties for 13 weeks.

Nutrient analyses, mycotoxin and FDA PAM 304 pesticide residue analyses were conducted on the corn lines to confirm their acceptability for use in the study. Event-specific PCR (polymerase chain reaction) and/or chain-of-custody records also confirmed the identities of the test, control and six reference control corn varieties. Using the nutrient analyses from each corn line, diets were formulated by Purina Mills, Inc. (PMI; St. Louis, MO) and Purina TestDiet (Richmond, IN) to be comparable in composition to PMI Certified Rodent Diet #5002. Formulated diets were analyzed to confirm that the specifications for a Certified Rodent Diet #5002 were met. Salt analysis tested the homogeneity of diet mixing. PCR confirmed the presence of the test event in the test diets and absence of the test event in control and reference control diets.

Male and female Sprague Dawley rats (20/sex/group) at approximately 6 weeks of age, were fed one of the following diets for 13 weeks: 1) diets containing 11% (w/w, low dose) or 33% (w/w, high dose) corn event MON 863 test grain, 2) diets containing 11% (w/w, low dose) or 33% (w/w, high- dose) nontransgenic control line LH82 x A634 grain, or 3) diets containing 33% (w/w) corn grain from six different reference varieties. The low-dose (11%, w/w) test and control diets were supplemented with 22% (w/w) nontransgenic corn from commercial sources to bring the total corn grain content in these diets to 33% (w/w), consistent with other test, control, and reference control diets.

PMI Certified Rodent Diet #5002 consumption was measured during pretest for one week (Week -1) to establish baseline food consumption for each animal. Diets containing the test, control and reference control corn grain were subsequently administered during the study period of Week 1 through Week 13. To assess the palatability of the diets, food consumption was recorded as follows: daily for Days 1, 2, 3, and once for 4 through 7 during pretest and Week 1. Food consumption was also measured weekly during Weeks 2 through 13 to assess potential test article-related effects. All animals were observed twice daily for mortality and moribundity. Body weight was recorded at weekly intervals for each animal. In Weeks 5 and 14, blood and urine were collected from 10 animals/sex/group for blood and urine

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chemistry, hematology, and urinalyses. Coagulation parameters were determined at the terminal blood collection only. In Week 14, all animals were sacrificed and necropsied. Tissues were collected and organs weighed as specified in the protocol. Selected tissues were examined microscopically from all animals in the high-dose test group and high-dose nontransgenic control group.

For quantitative measures, the test group was compared statistically with (1) its nontransgenic control counterpart and (2) the population of rats from the reference control groups fed the nontransgenic commercial corn varieties. Differences were considered statistically significant at the 5% level of significance ($p < 0.05$). The reference control diets were used to establish the range of responses from rats fed different nontransgenic corn varieties. The incidence of microscopic lesions in the control high-dose and the test high-dose groups was compared by sex using Fishers Exact Test.

There were no test article-related deaths or adverse clinical signs observed during the study. There were three unscheduled deaths among males, one nontransgenic high-dose control male, one reference control male, and one male in the high-dose test group. None of these deaths was attributed to treatment. Two females from two different reference control groups died immediately after blood collection in Week 5; their deaths were classified as accidental. Body weight gain and food consumption were similar in all groups throughout the study. Clinical pathology results (chemistry, hematology, coagulation and urinalyses) were similar across groups with only a few exceptions. The few statistically significant differences in clinical parameters that occurred were generally of small magnitude, were observed at the interim but not at the terminal bleed, were not dose related and/or were within +/- one standard deviation of the mean of the reference control groups. Organ weights and gross pathology findings were similar among test, control, and reference control groups. There were no gross or microscopic lesions attributed to a dietary regimen of high-dose concentration of test event MON 863.

The results of the 13-week subchronic feeding study show that rats fed diets containing corn event MON 863 grain responded similarly to rats fed diets containing the nontransgenic control LH82 x A634 grain and diets containing grain from reference control nontransgenic commercial corn varieties. There were no test article-related changes based on the evaluation of survival, clinical signs, body weights, body weight changes, food consumption, clinical pathology, organ weights, and macroscopic and microscopic pathology.

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1.0 Summary

This study was undertaken to compare the responses of rats fed diets containing grain derived from MON 863 corn to rats fed diets containing grain from the nontransgenic control line and a population of rats fed reference control diets containing grain from six different commercially available nontransgenic corn varieties for 13 weeks.

Nutrient analyses, mycotoxin and FDA PAM 304 pesticide residue analyses were conducted on the corn lines to confirm their acceptability for use in the study. Event-specific PCR (polymerase chain reaction) and/or chain-of-custody records also confirmed the identities of the test, control and six reference control corn varieties. Using the nutrient analyses from each corn line, diets were formulated by Purina Mills, Inc. (PMI; St. Louis, MO) and Purina TestDiet (Richmond, IN) to be comparable in composition to PMI Certified Rodent Diet #5002 . Diets were analyzed to confirm that the specifications for a Certified Rodent Diet #5002 were met. PCR confirmed the presence of the test event in the test diets and absence of the test event in control and reference control diets.

Male and female Sprague Dawley rats (20/sex/group) at approximately 6 weeks of age were fed one of the following diets for 13 weeks: 1) diets containing 11% (w/w, low dose) or 33% (w/w, high dose) corn event MON 863 test grain, 2) diets containing 11% (w/w, low dose) or 33% (w/w, high dose) nontransgenic control line LH82 x A634 grain, or 3) diets containing 33% (w/w) corn grain from six different reference varieties. The low-dose (11% w/w) test or control diets were supplemented with 22% (w/w) nontransgenic corn from commercial sources to bring the total corn grain content in these diets to 33% (w/w), consistent with other test, control, and reference control diets.

PMI Certified Rodent Diet #5002 consumption was measured during pretest for one week (Week -1) to establish baseline food consumption for each animal. Diets containing the test, control and reference corn grain were subsequently administered during the study period of Week 1 through Week 13. To assess the palatability of the diets, food consumption was recorded as follows: daily for Days 1, 2, 3, and once for 4 through 7 during pretest and Week 1. Food consumption was measured weekly during Weeks 2 through 13 to assess potential test article-related effects. All animals were observed twice daily for mortality and moribundity. Body weight was recorded at weekly intervals for each animal. In Weeks 5 and 14, blood and urine were collected from 10 animals/sex/ group for blood and urine chemistry, hematology, and urinalyses. Coagulation parameters were determined at the terminal blood collection only. In Week 14, all animals were sacrificed and necropsied. Tissues were collected and organs weighed as specified in the protocol. Selected tissues were examined microscopically from all animals in the high-dose test group and high-dose nontransgenic control line group.

For quantitative measures, the test group was compared statistically with (1) its nontransgenic control counterpart and (2) the population of rats from the reference control groups fed the nontransgenic commercial corn varieties. Differences were considered

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statistically significant at the 5% level of significance ($p < 0.05$). The reference control diets were used to establish the range of responses from rats fed different nontransgenic corn varieties. The incidence of microscopic lesions in the control high-dose and the test high-dose groups was compared by sex using Fishers Exact Test.

There were no test article-related deaths or adverse clinical reactions observed during the study. There were three unscheduled deaths among males, one nontransgenic high-dose control male, one reference control male, and one male in the high-dose test group. None of these deaths was attributed to treatment. Two females from different reference control groups died immediately after blood collection in Week 5; their deaths were classified as accidental. Body weight gain and food consumption were similar in all groups throughout the study. Clinical pathology results (chemistry, hematology, coagulation and urinalyses) were similar across groups with only a few exceptions. The few statistically significant differences in clinical parameters that occurred were generally of small magnitude, were not dose related and/or were within +/- one standard deviation of the mean of the reference control groups. Organ weights and gross pathology findings were similar among test, control, and reference control groups. There were no gross or microscopic lesions attributed to a dietary regimen of high-dose concentration of test event MON 863.

The results of the 13-week subchronic feeding study show that rats fed diets containing corn event MON 863 grain responded similarly to rats fed diets containing the nontransgenic control LH82 x A634 grain and diets containing grain from reference control nontransgenic commercial corn varieties. There were no test article-related changes based on the evaluation of survival, clinical signs, body weights, body weight changes, food consumption, clinical pathology, organ weights, and macroscopic and microscopic pathology.

2.0 Introduction

Monsanto Company has developed corn event MON 863, which protects corn plants against feeding damage from corn rootworm larvae (*Diabrotica* spp.), a major North American insect pest. Event MON 863 contains a coding sequence that expresses a variant of the wild-type Cry3Bb1 insecticidal protein from *Bacillus thuringiensis*. This study included the grain of the test event MON 863 and the nontransgenic control line LH82 x A634, which has background genetics representative of the test grain but does not express the Cry3Bb1 insecticidal protein.

3.0 Purpose

The purpose of this study was to compare parameters in Sprague Dawley rats fed a diet containing corn event MON 863 grain to (1) rats fed a diet containing grain from its nontransgenic control line LH82 x A634 and (2) a population of rats fed diets containing grain from six different nontransgenic commercial corn varieties as reference controls, representing a diversity of germplasm that were grown in different geographies.

4.0 Good Laboratory Practices (GLP) Compliance

The in-life phase of the rat feeding study was conducted in compliance with the appropriate provisions of the OECD (1981) Principles of Good Laboratory Practices and MHLW Good Laboratory Practices (GLP) Regulations. Compositional, pesticide, immunochemical and/or molecular analyses of grain samples and dietary compositional and pesticide residue analyses were performed according to U.S. EPA Good Laboratory Practice Standards 40 CFR Part 160. Mycotoxin analyses of grains were not conducted according to Good laboratory Practice Standards but were conducted under high scientific standards. The diets were prepared in an ISO 9002 certified facility. Stability analysis of the test diets was not performed in this study; however, Purina Mills has demonstrated that PMI Certified Rodent Diet #5002 is stable for at least six months. The diets prepared for this study were formulated with comparable ingredients to PMI Certified Diet #5002 and all diets were used within six months of manufacture.

5.0 Materials and Methods

5.1 Test Article:

The test article in this study was the grain produced from corn event MON 863, which contains a coding sequence that expresses a variant of the Cry3Bb1 insecticidal protein. MON 863 was grown in Kihei, Hawaii, U.S. during the 2000 growing season.

5.2 Control Article:

The control article used was the grain produced from the nontransgenic control line, LH82 x A634, which has the same background genetics representative of the test article but does not contain the *cry3Bb1* coding sequence. Control line LH82 x A634 was grown in Kihei, Hawaii, U.S. during the 2000 growing season.

5.3 Reference Control Articles:

The reference control materials used in this study included grain produced from six different commercial nontransgenic corn varieties. These reference control materials are designated in the rodent feeding study report (Appendix I) as follows: MON 847 Rep1 and Asgrow RX-770, from Monmouth, IL; LH235 x LH185, LH200 x LH172 and B73Ht x LH82 from Kaunakakai, HI; and Burrus BX-86 from Carlyle, IL. Information pertaining to the source and characterization of the reference control materials is provided in Appendix I.

5.4 Test, Control and Reference Substance Characterization

The test, control and reference control articles in this study were the corn grain. Prior to formulation of the study diets, the identity of the test grain was confirmed by the presence of the *cry3Bb1* coding sequence using an event-specific PCR. The absence of this coding sequence was confirmed in the control line LH82 x A634 grain using the same event-specific PCR method. Reference control grains were identified by chain of custody documentation. The test, control and reference control grains were analyzed at Covance Laboratories, Madison, WI for potential pesticide residues using the FDA PAM 304 pesticide screen and for key nutrients. The test, control and reference control grain materials were also analyzed for potential mycotoxin contaminants. The purpose of these tests (PAM 304 pesticides and mycotoxins) was to ensure that the grain samples used in the feeding study did not contain unacceptable levels of mycotoxins or pesticide residues that might interfere with the results

of the study.

5.5 Preparation of Diets:

The vehicle for administering the corn grain to rats was a formulated diet. Purina TestDiet (Richmond, IN) was the facility used to formulate nutritionally balanced diets containing the test, control or reference (T/C/R) corn grain. Purina TestDiet is a unit of PMI that makes specialized diets. An animal nutritionist at PMI used the results of the nutrient tests conducted at Covance, WI to prepare diet formulas using computer programs. Formulas for nutritionally balanced diets for rats were transmitted to Purina TestDiet for preparation of diets. The diets were formulated to be comparable in composition to PMI Certified Rodent Diet #5002. Test, control, and reference control variety grains were ground and added to the appropriate diets at levels of approximately 33%, (w/w) which is the concentration of corn generally prescribed in the aforementioned certified rodent diet. Test and control grain was also added to diets formulated at 11% (w/w) to determine if there was a dose response of any potential effects that might be observed at the 33% (w/w) dietary level. Nontransgenic commercial reference grain was added at 22% (w/w) to these 11% (w/w) test event MON 863 and control line LH82 x A634 diets to bring the total corn content up to 33% (w/w), the targeted concentration in rodent diets. All diets were sampled at Purina TestDiet. Samples were sent to Covance Laboratories (Madison, WI) for analysis to confirm that the formulated diets met the nutritional, mycotoxin and pesticide residue specifications for PMI Certified Rodent Diet #5002. Samples were collected for determining homogeneity by measuring salt (NaCl) distribution in the diet batches. Samples were also sent to Monsanto Company, Chesterfield, MO, for confirmation of the presence of or absence of the test event MON 863 in the test, control and reference control diets by event-specific PCR.

5.6 Experimental Design:

Rats were assigned to groups in a completely random manner. The outline of the dietary regimen of the groups used in the study is provided in the following table:

Corn Line (% w/w)	Animals/Sex
MON 863 - Test (11%)	20
MON 863 - Test (33%)	20
LH82 x A634 - Control (11%)	20
LH82 x A634 - Control (33%)	20
MON 847 Rep1 - Reference (33%)	20
Asgrow RX-770 - Reference (33%)	20
LH235 x LH185 - Reference (33%)	20
LH200 x LH172 - Reference (33%)	20
B73Ht x LH82 - Reference (33%)	20
Burrus BX–86 – Reference (33%)	20

5.7 Test Animals and Observations:

Sprague Dawley Crl: CD (SD)IGS BR rats were at least six weeks of age when the study started. Animals were observed twice daily for adverse clinical signs. Individual physical examinations and body weight determinations were conducted each week.

During pretest (Week -1), rats were fed PMI Certified Rodent Diet #5002, and food consumption was measured for each animal on Days 1, 2 and 3, and once over Days 4 through 7. This was done to provide baseline food consumption data. Test, control and reference control diets were presented on study Day 1. Food consumption was measured on study Days 1, 2, 3, and once over Days 4 through 7 to assess the palatability of formulated diets and compare with basal diet intake. Thereafter, food consumption of all groups was measured weekly from Weeks 2 through 13 for potential treatment effects.

Clinical pathology parameters (hematology, clinical chemistry and urinalyses) were measured for 10 rats/sex/group in study Week 5 and at the end of the study. Blood coagulation parameters were evaluated at the end of the study. After Week 13, all animals were sacrificed and necropsied. Organ weights were recorded at necropsy for adrenals, brain, heart, kidneys, liver, spleen and gonads. A complete set of tissues was collected from each animal and preserved in formalin. Tissues from the major organ systems (adrenals, brain, colon, duodenum, heart, ileum, jejunum, kidneys, liver, lymph node [mesenteric], ovaries, pancreas, rectum, spleen, stomach, testes and thyroid/parathyroid) were examined microscopically from all high-dose test and high-dose control animals.

5.8 Statistical Methods:

The quantitative measurements of groups fed test diets were compared with (1) groups fed the control diets and (2) the reference control population fed the six reference control corn variety diets. The data for each sex were fit by a simple one-way analysis of variance model and specific treatment combinations were compared using contrasts. Differences were considered statistically significant at $p < 0.05$, when both the ANOVA and contrasts comparison were statistically significant.

Quantitative measures (body weights, cumulative body weight changes, food consumption, clinical pathology data and organ weight data) were examined for variance heterogeneity, outliers and other anomalies that might compromise the validity of the standard analysis measurements described previously. If such conditions existed, they were accommodated by transforming data or by using resistant or non-parametric analogs of the standard analyses. All such accommodations were documented.

The reference control groups were used to determine whether statistically significant differences between the control and test groups were test-article related. For instance, when a parameter from the high-dose test group was statistically significantly different from the high-dose control group, but was within the range of the population of reference controls, the difference was not considered to be test-article related.

Sprague Dawley Crl:CD (SD) IGS BR is a registered trademark of Charles River Laboratories.

The incidences of microscopic findings were analyzed by sex using Fisher's Exact Test. Differences were considered statistically significant at $p<0.05$.

6.0 Results

6.1 Diet Analysis:

Event-specific PCR analysis confirmed the identity of the test and control grain prior to their selection for use in diet preparation. Compositional, mycotoxin and FDA PAM 304 pesticide residue analyses of grain samples selected for the study established that these grains were suitable for feeding to rats. MON 863 event-specific PCR analysis of samples of each of the formulated diets confirmed that only the test diets contained event MON 863 grain, whereas the control and reference control diets did not contain event MON 863. Compositional, mycotoxin and pesticide residue analyses confirmed that formulated diets met the specifications for PMI Certified Rodent Diet #5002, with one exception. The limit of detection of the analytical method in diets used by Covance Laboratories for chlordane was higher (250 ppb) than the PMI maximum specification of 50 ppb for Certified Rodent Diet #5002; therefore the specification could not be confirmed. This was not considered to have an impact on the study.

6.2 Animal Observations:

There were no treatment-related deaths or adverse clinical reactions observed in the study. There were three unscheduled deaths among males: one each in the high-dose control, reference control, and high-dose test group. None of these deaths was attributed to treatment. Two females from different reference control groups died immediately after blood collection in Week 5; their deaths were classified as accidental. Clinical observations were typical of Sprague Dawley rats of this age.

Body weights and cumulative body weight changes of male and female rats fed test diets were generally similar to those of rats fed the nontransgenic control and reference control diets. Food consumption values indicated no palatability effects due to the diets containing the test, control or reference control grain. There was no indication of excess feed spillage. For the duration of the study, food consumption was similar in all groups.

6.3 Clinical Pathology:

Hematology and coagulation parameters for the test groups were generally comparable to the control and reference control groups. The few differences that were observed were generally of small magnitude, were not dose related -- that is, observed in the low dose but not the high dose -- or were observed at the interim but not the terminal blood collection, and/or were not consistently observed when compared to both the nontransgenic control and reference control groups. For example, at Week 5, the hemoglobin level of high-dose test males was outside the range of the reference control groups, but significantly lower ($p<0.05$) than the concurrent control. However the hemoglobin level of high-dose males was higher than the low-dose males, thus demonstrating no dose response. No similar statistical difference was seen at Week 14. At Week 14, the mean basophil count, lymphocyte count and total white cell count values were slightly but statistically significantly higher in the male group fed the high-dose test diet than males fed the high-dose control and reference control diets.

However, the increases were small and a similar response did not occur in females. These changes are not attributed to dietary administration of the test article. No other hematology differences occurred.

Clinical chemistry results of the test groups were generally similar to the control and reference control groups. Only the triglyceride value was statistically significantly higher in female rats fed the high-dose test diet relative to high-dose control and the reference control groups at Week 5. No similar response was seen in males at either sampling interval. The increase in mean triglycerides in test high females at Week 5 was small and the mean value was within +/- one standard deviation of the mean of the reference control groups lower than the value of the low-dose control group. There were no other chemistry parameters associated with this change that would indicate a pattern of alteration. This slight difference was not considered to be test-article related because of lack of dose response, and the inconsistency of the changes across time.

6.4 Organ Weights:

No test-article related differences were observed in organ weights, organ weights relative to body or organ weights relative to brain weights. Organ weights were similar in all groups.

6.5 Gross and Microscopic Pathology:

Gross pathology changes were randomly distributed among all groups, were low in incidence, and were of the kind commonly observed in rats of this age and strain. None was considered to be test-article related. Histomorphologic examination of tissues representing major organs systems found no test-article related alterations. The statistically significant lower incidence of a common kidney lesion (tubular mineralization) in high-dose test females compared to high-dose control females is of no biological importance and not considered to be test-article related.

7.0 Conclusion

The results of the 13-week subchronic feeding study show that rats fed diets containing corn event MON 863 grain responded similarly to rats fed diets containing the nontransgenic control LH82 x A634 grain and diets containing grain from reference control nontransgenic commercial corn varieties. There were no test article-related changes based on the evaluation of survival, clinical signs, body weights, body weight changes, food consumption, clinical pathology, organ weights, and macroscopic and microscopic pathology.