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REPORT SUMMARY

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Sponsor Summary of 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 non-transgenic 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 non-transgenic control line and a population of rats fed reference control diets containing grain from six different commercially available non-transgenic 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.<sup>®</sup> 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-

<sup>®</sup> Certified Rodent Diet # 5002 is a registered trademark of Purina Mills, Inc.

dose) non-transgenic 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) non-transgenic 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 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 non-transgenic control group.

For quantitative measures, the test group was compared statistically with (1) its non-transgenic control counterpart and (2) the population of rats from the reference control groups fed the non-transgenic 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 non-transgenic 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 non-transgenic 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 non-transgenic control LH82 x A634 grain and diets containing grain from reference control non-transgenic 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.

APPENDIX 7

Statistical Report  
Statistical Report Quality Assurance Statement

Covance Study No. 6103-293; Monsanto Study No. CV-2000-260  
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**Statistical Report**

**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**

*Covance Study No. 6103-293; Monsanto Study No. CV-2000-260*

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### Data Description

The data consisted of male and female rat body weight and body weight change measurements, food consumption measurements, clinical chemistry measurements, hematology measurements, urinalysis measurements, organ weight, and histopathology findings in animals fed diets containing grain from MON 863 corn, non-transgenic control LH82 X A634 (with background genetic representative of the test line MON 863 but not genetically enhanced), and six commercial lines (MON 847 Rep 1, Asgrow RX-770, LH235 X LH185, LH200 X LH172, B73HT X LH82 and Burrus BX-86). In addition MON 863 and LH82 X A634 were present at either 11% or 33% in the diet while the commercial lines were at 33%. Each group contained 20 rats/sex.

The data were provided by Covance as SAS<sup>®</sup> (1999-2001) transport datasets. These data were saved as SAS data files and analyzed with SAS, release 8.2. A listing of the original data is included in the statistical documentation of the data.

### Statistical Analysis

Separate analyses were done for the males and the females. The test diets were compared using a one-way analysis of variance model, i.e.,

$$y_{ij} = \mu + \tau_i + \epsilon_{ij}$$

where

- $y_{ij}$  is the value of the response for test diet  $i$  and rat  $j$
- $\mu$  is the overall mean
- $\tau_i$  is the mean for test diet  $i$
- $\epsilon_{ij}$  is the random error for rat  $j$  fed test diet  $i$ .

Levene's test, using a  $p$ -value  $< 0.01$  for statistical significance, was conducted to check for equality of variances. If the equality of variance assumption was rejected then the analysis of variance was done on the rank of the measured response rather than the actual response. Though the normality assumption for the statistical test might be violated due to the abnormal distribution or a few extreme values for some data, approximate normality could be satisfied for the means of the contrasts from samples of 20 or 10 for each treatment. Three contrasts among the test diets were constructed and tested. These contrasts were: MON863 at 11% versus LH82xA634 at 11% (CL 11% vs TL 11%); MON 863 at 33% versus LH82xA634 at 33% (CH 33% vs TH 33%); MON 863 at 33% versus the average of the six commercial lines (TH 33% vs REF) at 33%. The level of significance used for the overall ANOVA and all contrasts was  $p$ -value  $< 0.05$ .

Tables 1 - 7 give means, standard deviations and  $p$ -values for the overall analysis of variance and the three contrasts. With only a few missing data points, the number of rats

for each of the diets is 20 but for clinical chemistry, hematology and urinalysis data, results were recorded for 10 rats/sex/diet.

#### Qualitative Responses

The microscopic pathology data were analyzed using Fishers Exact Test for a 2x2 contingency table. A separate analysis was done for each sex. The incidences of microscopic findings, for each tissue and lesion type, were tabulated for the 33% MON 863 and 33% LH82xA634 groups. The results of the statistical analysis are in Table 8.

#### **Results**

#### Quantitative Responses

There were instances where statistical significance was observed. The procedure for assessing the statistical analysis results was two-fold. First, if the overall analysis of variance was significant ( $p$ -value  $< 0.05$ ) then the contrasts between the test diet at 33% and the pooled commercial diets were investigated for significance, i.e., the one-degree of freedom contrasts were evaluated using a  $t$ -test procedure. This is similar to the protected LSD since the contrasts were evaluated in the presence of significance of the overall analysis of variance. Second, the significant contrasts were evaluated with respect to the means for the commercial lines. If the means of the test diet at 33% were within the range of means for the commercial diets, then the significance was not deemed to be biologically meaningful.

Using the above criteria, there was no significant difference detected for data in food consumption, body weight, and organ weight. There were 19 findings that satisfied above criteria (both overall test and the comparison between the test line and the mean of the commercial group were significant, and in addition the test line was outside of the range of the commercial group), 2 in body weight change, 6 in hematology traits, 8 in clinical chemistry measurements, 3 in urinalysis measurements. In 14 out of all 19 cases, the obvious reason for the significance was due to both MON 863 and LH82xA634 being either outside of or near the border of the range of the commercial lines in the same direction (high or low limit of the range) and exceptions included, Male Hemoglobin - Week 5, Male Hematology White Blood Cell Count - Week 14, Male Hematology Lymphocytes - Week 14, Male Hematology Basophils Absolute - Week 14, and Female Chemistry Triglycerides - Week 5. All significance was sporadic and no consistent significance was found across time period for any measurement.

#### Qualitative Responses

Only 1 (Female, Kidney, Tubule - Mineralization) out of 68 histopathology incidence frequency comparisons between MON 863 and LH82xA634 at 33% of the diet in Table 8 were statistically significant at the 0.05 level by Fisher's exact test while on average 3 false positives would be expected among 68 comparisons. The  $P$ -value of the significance is 0.031 and only marginal significant at the 0.05 level.



**Conclusions/Summary**

Although a few statistically significant differences between MON 863 and the non-transgenic control LH82xA634 (with background genetic representative of MON 863 but not genetically enhanced) were showed in the data, no patterns could indicate any effects were test article related.

**References**

SAS<sup>®</sup>, Release 8.2 on-line documentation, 1999-2001 SAS Institute, Inc., Cary, NC, USA.

### 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

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