Review of 13-Week Rat Feeding Study with MON863 Corn

Background

Monsanto applied to FSANZ to approve food from MON863 corn in December 2002. The approval for food from MON863 corn came into effect in December 2003 in Australia and April 2004 in New Zealand. FSANZ has recently been made aware of a 13-week rat feeding study with MON863 corn, which was not previously assessed by FSANZ. Questions have been raised in relation to the results of this study by the French Commission du Génie Biomoléculaire (CGB) during its assessment in Europe, as a consequence, FSANZ has assessed all the available data that Monsanto has provided.

Assessment of MON863 corn in Europe

Monsanto Europe made an Application in the European Union for MON863 corn and corn hybrid MON863 x MON810 in July 2002. The Application was lodged with the German Competent Authority and was subsequently assessed by the Robert Koch Institut (RKI) in Berlin. Following Member State review of the RKI assessment, the French Competent Authority requested subchronic toxicity data for rats fed the grain of MON863 corn. Monsanto Europe subsequently provided a 13-week rat feeding study to the French Competent Authority. In November 2003, the French Commission du Génie Biomoléculaire (CGB) asked Monsanto for clarification of the significance of some of the results from this study. In response, Monsanto provided supplementary analyses of the study findings. The GMO Panel of the European Food Safety Authority (EFSA) has examined the results of the 13-week rat feeding study and concluded that the results of this study do not indicate adverse effects from consumption of maize line MON863. The GMO Panel subsequently concluded that the "the placing on the market of MON863 is unlikely to have an adverse effect on human and animal health or the environment in the context of its proposed use." The Opinion was adopted on 2 April 2004.

Data provided to FSANZ by Monsanto

Monsanto have provided the following material to FSANZ[1]:

1. Lemen, J.K., Hammond, B.G., Riordan, S.G., Jiang, C. and Nemeth, M. (2002). 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. Monsanto Company, 18 December 2002 (Report No. MSL-18175).

2. Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safety of foods and food ingredients derived from insect-protected genetically modified maize MON863 and MON863 x MON810, for which a request for placing on the market was submitted under Article 4 of the Novel Food Regulation (EC) No 258/97 by Monsanto (Question No EFSA-Q-2003-121), 2 April 2004.

[1] The full report of Monsanto Company Study CV 2000-260 was not available to FSANZ.

3. Hammond, B.G. and Ward, D.P (2004). Supplemental Analysis of Selected Findings on the Rat 90-Day Feeding Study with MON863 Maize Report MSL-18175. Monsanto Company, 24 May 2004.

4. Hard, G.C. and Terron, A. (2004). Retrospective Evaluation of Renal Tissues and Data from Monsanto Company Study CV-2000-260 (MSL-18175): A 13-Week Rat Feeding Study with MON863 Corn. Seventh Wave Pathology and Biotechnical Solutions LLC, 11 August 2004.

Evaluation of the 13-Week Comparison Study in Rats

Study Conduct

The study design is an adaptation of OECD Guideline No. 408 for a Repeated Dose 90-day Oral Toxicity Study in Rodents. The performing laboratory was Covance Laboratories (Virginia, United States). Groups of Crl:(SD)CD IGS BR rats (20/sex/group) were fed diets formulated with either MON863 corn or conventional corn. Conventional corn was either the parental control for MON863 corn, or commercially available conventional corn varieties.

Two parental control groups were fed diets formulated to contain either 11% (low dose) or 33% (high dose) parental control corn. The 11% parental control diet was supplemented with 22% corn from commercial conventional varieties. Two test groups were fed diets formulated to contain 11% (low dose) or 33% (high dose) MON863 corn, with the low dose diet being supplemented with 22% commercial conventional corn. Six reference control groups were included in the study and were fed diets containing 33% of six commercially available conventional corn varieties. The additional reference control groups were included to establish a normal range of values for all the parameters measured. The composition of the control and tests diets were analysed (data provided) and determined to be compositionally equivalent.

Diets containing the test, control and reference control corn were provided *ad libitum* for a period of 13 weeks. All animals were observed twice daily for mortality and moribundity. Body weight and food consumption 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, haematology, and urinalyses. Coagulation parameters were determined at the terminal blood collection only. In Week 14, all animals were killed and necropsied. Tissues were collected and organs weighed. Selected tissues were examined microscopically from all animals in the high-dose test group and high-dose parental control group.

Summary of Study Findings

No treatment-related deaths or adverse clinical signs were observed during the study and body weight gain and food consumption were similar across all groups (data not provided). Clinical pathology results (chemistry, haematology, coagulation and urinalyses) showed no statistically significant differences between parental and MON863 groups except for (i) a small statistically significant increase in white blood cell (WBC) and lymphocyte counts in high dose males at week 14; (ii) a small statistically significant decrease in reticulocyte count

in high dose females at week 14; and (iii) a small statistically significant increase in glucose levels in low and high dose females at week 14.

Organ weights and gross pathology showed no significant differences between parental and MON863 groups except for (i) a small statistically significant decrease in kidney weights in high dose males; and (ii) a non-statistically significant increase in some histopathological changes in both sexes.

These results are reviewed in detail below.

White blood cell (WBC) and lymphocyte count

The increase in WBC count in males at week 14 is largely attributed to an increase in the lymphocyte count, as there were no significant differences observed with other WBCs (neutrophils, basophils, eosinophils, and monocytes) (data not provided). The spleen is not enlarged in MON 863-fed males and there were no histopathological changes in this organ or in the lymph nodes (data not provided). There was no statistically significant difference in lymphocyte counts for females. These differences are not considered to be biologically significant since they fall within the standard deviation of the reference control population (Table 1 and Figure 1).

Table 1: Lymphocyte counts for MON863-fed and control rats at week-14

Parameter (10^3/μL)	**MON863 (range)**	**Parental Control (range)**	***P*-Value**	**Reference Controls**	**Historical Controls**
♂ Lymphocytes (11% dose level)	6.7 (4.1–8.0)	5.9 (3.2-9.1)	0.283		
♂ Lymphocytes (33% dose level)	8.8 (7.0-11.3)	7.2 (4.2-10.9)	0.042*	3.5-11.4	9.2 ± 1.6
♀ Lymphocytes (11% dose level)	7.1 (5.3-9.3)	5.7 (2.6-7.6)	0.072		
♀ Lymphocytes (33% dose level)	5.7 (3.6-10.0)	4.7 (2.2-6.8)	0.16	3.0-10.4	

* Anova group analysis significant $p<0.05$

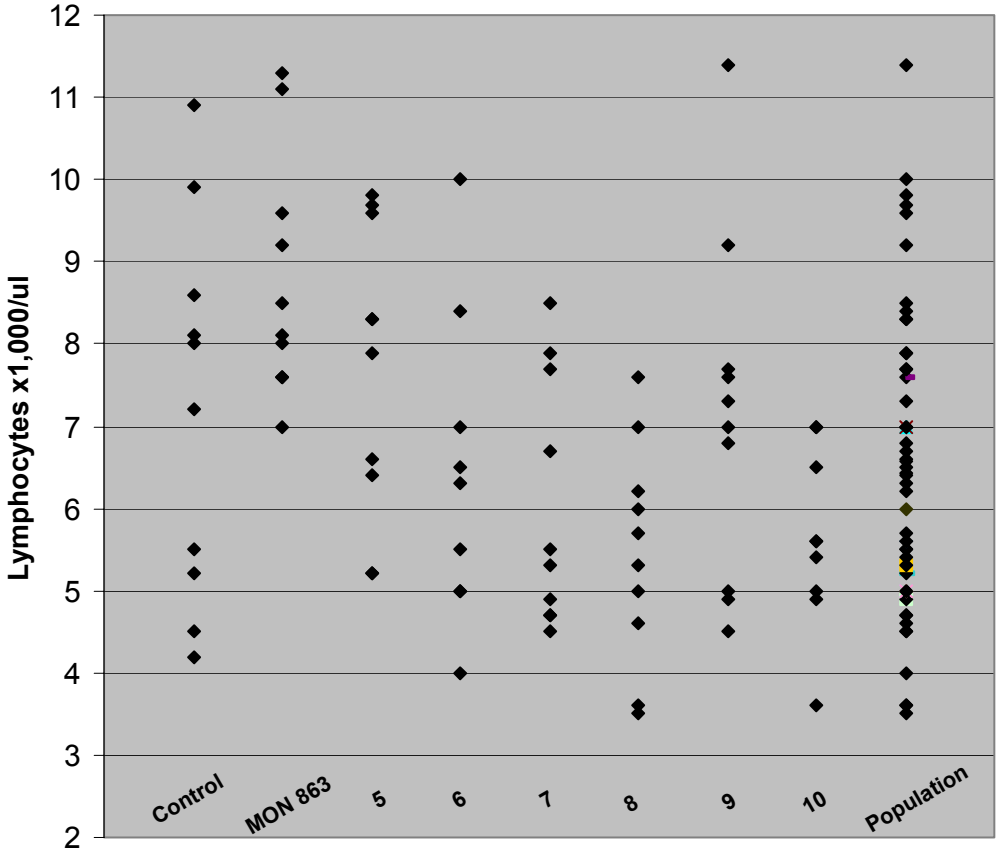

Figure 1: Week-14 individual male lymphocyte counts for MON863, parental control (control) and six reference control groups (5-10). The reference control group data are also presented grouped (population).

Reticulocyte count

The decrease in reticulocyte count for females is not accompanied by a reduction in RBC count or haematocrit (data not provided). Male reticulocyte counts were all within normal limits and the female reticulocyte counts were all within parental and reference control ranges (see Table 2 and Figure 2), therefore, these differences are not considered to be biologically significant.

Table 2: Reticulocyte counts for MON863-fed and control rats at week-14

Parameter (10^6/µl)	**MON863 (range)**	**Parental Control (range)**	***P*-Value**	**Reference Controls**	**Historical Controls**
♂ Reticulocytes (11% dose level)	0.07 (0.01-010)	0.06 (0.02-0.12)	0.543		
♂ Reticulocytes (33% dose level)	0.06 (0.02-0.14)	0.07 (0.01-0.14)	0.589	0.01-0.17	
♀ Reticulocytes (11% dose level)	0.06 (0.01-0.12)	0.09 (0.04-0.19)	0.104		
♀ Reticulocytes (33% dose level)	0.04 (0.01-0.10)	0.09 (0.02-0.15)	0.016*	0.02-0.20	1.10 ± 0.08

* Anova group analysis significant $p<0.05$

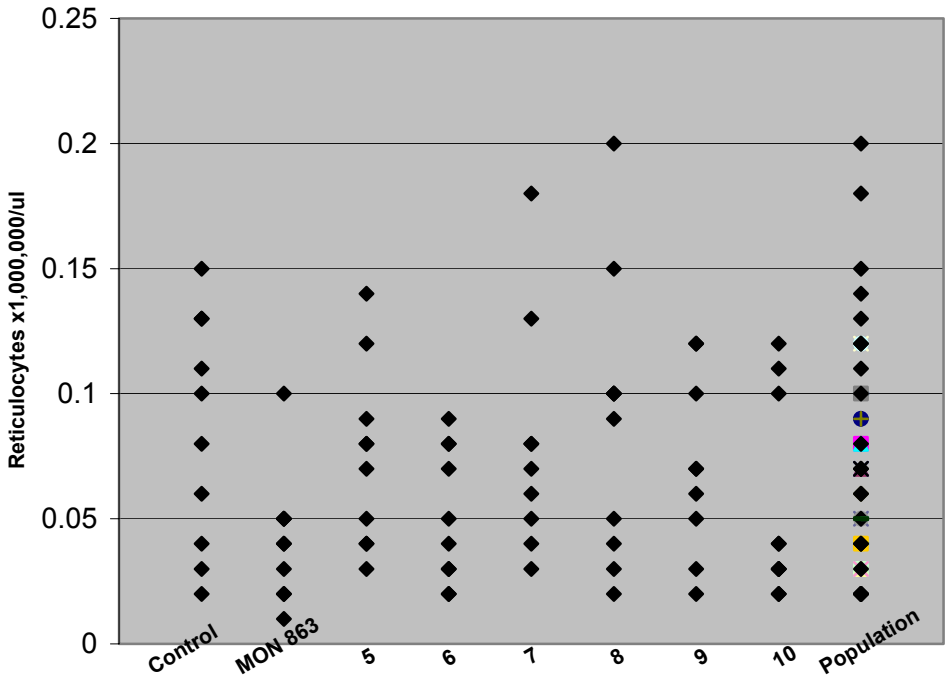

Figure 2: Week-14 individual female reticulocyte counts for MON863, parental control (control) and six reference control groups (5-10). The reference control group data are also presented grouped (population).

Serum glucose level

The range of values measured overlaps the range of values for the parental control and were well within the range of values for the reference control groups (Table 3 and Figure 3). Group mean values were also only slightly higher than group mean values from historical controls from the testing laboratory. There was no statistically significant difference in serum glucose levels for males.

Table 3: Serum glucose level for MON863-fed and control rats at week-14

Parameter (mg/dl)	**MON863 (range)**	**Parental Control (range)**	***P*-Value**	**Reference Controls**	**Historical Controls**
♂ Serum Glucose (11% dose level)	105 (93-118)	108 (98-117)	0.378		
♂ Serum Glucose (33% dose level)	116 (100-168)	109 (97-120)	0.084	90-126	
♀ Serum Glucose (11% dose level)	113 (94-133)	103 (88-115)	0.041*		
♀ Serum Glucose (33% dose level)	116 (103-126)	105 (97-122)	0.021*	93-143	111 ± 10

* Anova group analysis significant $p<0.05$

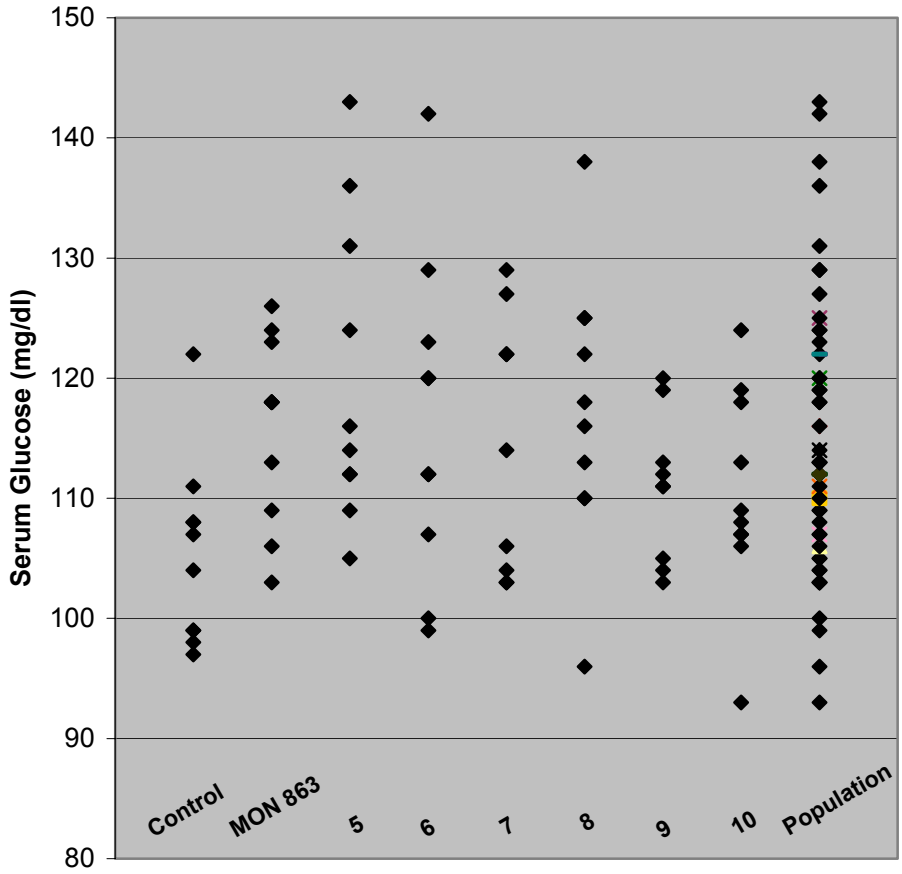

Figure 3: Week-14 individual female serum glucose levels for MON863, parental control (control) and six reference control groups (5-10). The reference control group data are also presented grouped (population).

Renal changes

All individual MON 863-fed male kidney weight values fall within the range of reference control values (Table 4 and Figure 4). There were no changes in the kidney weights of females and no changes in other organ weights (data not provided). There were no changes in blood urea nitrogen and creatinine levels in male kidneys, which were reported to be within published normal ranges. An expanded peer review was conducted to independently review the renal effects (urinalysis, urine chemistry, organ weight, and histology). Some histopathological changes were observed in the kidneys from both control and test males and females, however all changes were of minimal or mild severity and not treatment-related.

Table 4: Kidney weights for MON863-fed and control rats at week-14

Parameter (g)	MON863 (Range)	Parental Control (Range)	*P*-Value	Reference Control
♂ Kidney wt (11% dose level)	3.24 (2.49-3.96)	3.35 (2.90-3.94)	0.324	
♂ Kidney wt (33% dose level)	3.20 (2.65-3.90)	3.45 (2.93-4.02)	0.034*	2.29-4.32
♀ Kidney wt (11% dose level)	1.99 (1.59-2.37)	1.94 (1.68-2.21)	0.365	
♀ Kidney wt (33% dose level)	1.95 (1.66-2.26)	1.90 (1.63-2.28)	0.446	1.53-2.43
♂ Kidney % bw (11% dose level)	0.68 (0.61-0.78)	0.69 (0.58-0.75)	0.743	
♂ Kidney % bw (33% dose level)	0.67 (0.59-0.75)	0.71 (0.59-0.87)	0.046*	0.55-0.86
♀ Kidney % bw (11% dose level)	0.75 (0.69-0.87)	0.76 (0.65-0.90)	0.590	
♀ Kidney % bw (33% dose level)	0.73 (0.58-0.92)	0.74 (0.63-0.88)	0.636	0.62-0.96

* Anova group analysis significant p<0.05

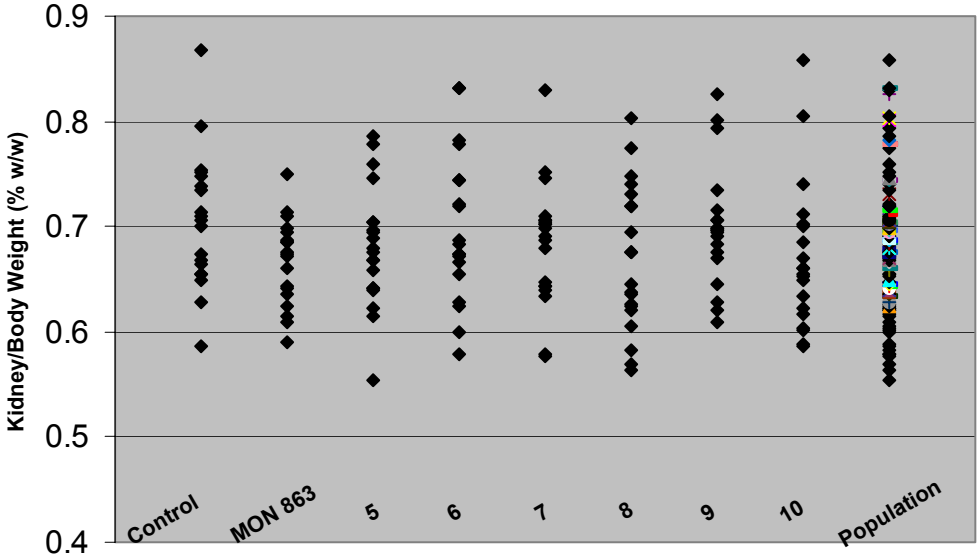

Figure 4. Individual male kidney weights as a percentage of terminal body weight for the MON 863, parental control (control), and the six reference control groups (5-10). The reference control group data are also presented grouped (population).

Overall histopathological changes

The only histopathological finding that reached statistical significance was a reduced incidence of renal tubular mineralization in high dose females (not an adverse effect), which was not confirmed by the independent pathologists. In general, the incidence of microscopic

changes in the MON863-fed rats was within the range of incidence for the historical controls. The exception to this was the incidence of liver necrosis and stomach glandular dilatation in MON 863-fed male rats, which was higher than that observed in both the parental and historical control groups (see Table 5). There are no corresponding increases in male liver enzyme levels or changes in liver weight (data not provided). Supplemental historical control incidence data by the performing laboratory (Covance Laboratories, data not provided) shows the incidence of male liver necrosis to range from 0-10% and for stomach glandular dilatation to range from 0-50%.

Table 5. Incidence of selected microscopic findings compared to historical control (HC) groups from five additional Monsanto 90-day maize feeding studies.

Microscopic Finding	MON 863 33%	Parental Control	HC-1	HC-2	HC-3	HC-4	HC-5
Kidney, tubule mineralization - ♀	2	9	9	5	6	1	2
Liver, necrosis - ♂	3	0	0	0	1	0	0
Stomach, glandular dilatation - ♂	4	1	0	0	0	0	0

Discussion and conclusions

In the study, rats were fed a diet containing either 11% or 33% (w/w) MON863 corn, and no treatment related effects were observed on growth, food consumption, morbidity, and moribundity compared to rats fed conventional corn. Haematology, clinical chemistry, urinalyses, and organ weights did not reveal any treatment related effects, except for a few small statistically significant differences in white blood cell and reticulocyte count and serum glucose levels between the test and control groups. A review of these findings was therefore undertaken to determine if there is any basis for concluding that the observed differences are treatment related.

It is important to note that the study, while based on the protocol for a sub-chronic toxicity study, is a comparative feeding study, with different varieties of corn. As such, its overall usefulness in assessing the safety of MON863 corn or its constituents is limited because of the limits on the amount of test material (in this case MON863 corn) that can be incorporated in an animal's diet, without creating a nutritional imbalance. In this particular study, the highest level of incorporation in the diet of MON863 corn was 33%. The ability of feeding studies to detect adverse effects from a constituent present in a food product will be largely dependent on the intrinsic toxicity of any such constituent and whether it is present in the food in a sufficient amount to induce toxicity under the conditions of the study. Notwithstanding these limitations, and providing the study has been well designed and executed, the absence of any adverse effects may however provide additional assurances of safety.

Overall, the study appears to have been well designed and executed. There is no evidence of any nutritional imbalances that would complicate interpretation of the study results.

These data do not indicate any adverse effects, which can be related to MON863 corn or its constituents. The most compelling evidence for this is the comparison of results for test group individuals against those for the parental, reference and historical controls. This clearly demonstrates that the observed differences in lymphocyte and reticulocyte counts, serum glucose levels, and kidney weights represent normal biological variability. This interpretation is supported by other observations, which indicate there is no underlying evidence of organ pathology to accompany the haematology, serum chemistry and kidney weight differences and no consistency in response between the sexes. In terms of the increased incidence of histopathological changes in the MON863-fed rats, none of these findings were statistically significant, their incidence is well within the range for historical controls, and none of the changes observed were correlated with changes in measured clinical parameters.

In conclusion, the observed differences are consistent with normal physiologic variation and are not related to the consumption of MON863 corn. The observed histopathological changes are similarly unremarkable for rats of this strain and age. Therefore, the results of this study do not indicate adverse effects from the consumption of MON 863 corn.