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Results of a 90-day safety assurance study with rats fed grain from corn borer-protected corn

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Abstract

The results of a 90-day rat feeding study with grain from MON 810 corn (YieldGard® Cornborer – YieldGard Cornborer is a registered trademark of Monsanto Technology, LLC) that is protected against feeding damage from corn and stalk boring lepidopteran insects are presented. Corn borer protection was accomplished through the introduction of *cry1Ab* coding sequences into the corn genome for in planta production of a bioactive form of Cry1Ab protein. Grain from MON 810 and its near-isogenic control was separately formulated into rodent diets at levels of 11% and 33% (w/w) by Purina Mills, Inc. (PMI). All diets were nutritionally balanced and conformed to PMI specifications for Certified LabDiet® (PMI Certified LabDiet 5002 is a registered trademark of Purina Mills, Inc.) 5002. There were a total of 400 rats in the study divided into 10 groups of 20 rats/sex/group. The responses of rats fed diets containing MON 810 were compared to those of rats fed grain from conventional corn varieties. Overall health, body weight, food consumption, clinical pathology parameters (hematology, blood chemistry, urinalysis), organ weights, and gross and microscopic appearance of tissues were comparable between groups fed diets containing MON 810 and conventional corn varieties. This study complements extensive agronomic, compositional and farm animal feeding studies with MON 810 grain, confirming that it is as safe and nutritious as grain from existing commercial corn varieties.

Keywords: Corn: genetically modified; Corn borer protected; MON 810; Rat feeding study

1. Introduction

Global regulatory authorities require that food derived from crops produced through biotechnology be *as safe as* food produced from conventionally bred crops. There must be "reasonable certainty that no harm will result from intended uses under the anticipated conditions of consumption" (OECD, 1993).

The World Health Organization (WHO, 1995), the United Nations Food and Agricultural Organization (WHO, 1991; FAO, 1996), the Organization for Economic Cooperation and Development (OECD, 1993, 1997), the Codex Alimentarius Commission (Codex, 2003) and the European Food Safety Agency (EFSA, 2004a) established a safety assessment process to assure that foods produced from biotechnology-derived products are *as safe as* food produced from conventionally bred crops. This assessment process considers two main categories of potential risk; those related to the properties and function of the introduced protein(s), and those resulting from insertion of the introduced gene(s) into the plant genome that might

Abbreviations: ANOVA, analysis of variance; APHIS, Animal and Plant Health Inspection Service; *Bt*, *Bacillus thuringiensis*; ELISA, enzyme-linked immunosorbent assay; EU, European Union; EPA, Environmental Protection Agency; FAO, Food and Agricultural Organization; fl, femtoliters; GLP, Good Laboratory Practices; NOEL, no-effect level; OECD, Organization for Economic Cooperation and Development; PCR, polymerase chain reaction; PMI, Purina Mills International; ppb, part-per-billion; SD, standard deviation; USDA, United States Department of Agriculture; WHO, World Health Organization; US, United States; w/w, weight/weight.

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theoretically cause unintended (pleiotropic) effects. The risk assessment for a biotechnology-derived crop is a comparative safety assessment using conventional food with a history of safe consumption as the reference point for all comparisons. The outcome of this assessment is to determine whether the genetically modified crop is comparable to the existing conventionally bred crop. Newly introduced proteins are subject to a separate safety assessment.

MON 810 corn (hereafter referred to as MON 810), marketed under the brand name YieldGard®.[1] Cornborer, was produced by insertion of a DNA sequence that encodes a bioactive form of *Bacillus thuringiensis* (*Bt*) Cry1Ab protein. The Cry1Ab protein is selectively toxic to the European corn borer (ECB, *Ostrinia nubilalis*), the southwestern corn borer (SWBC, *Diatraea grandiosella*) and the pink borer (*Sesamia cretica*). Protection against corn borer damage improves yields and reduces the need for chemical insecticide use (James, 2003). Reduced corn borer feeding on ears also decreases infection by toxigenic fungi such as *Fusarium* species that are present in the environment wherever corn is grown (Miller, 2001) and enter kernels damaged by insect feeding. Certain *Fusarium* species produce fumonisin mycotoxins that cause a variety of toxic effects in farm animals and may be harmful to humans (CAST, 2003; Marasas et al., 2004). MON 810 varieties have been shown to have lower fumonisin levels in the grain based on field studies in several countries (Munkvold et al., 1999; Bakan et al., 2002; Hammond et al., 2004a). Since its commercial introduction in the United States in 1996–2002, *Bt* corn has been grown on approximately 43 million hectares worldwide (James, 2003). Some of this corn grain is used in human food production. In the United States, approximately 2% (186 million bushels) of corn grain produced in 2001 (9507 million bushels) was used in human food (cereals) and other products (Leath, 2003). Approximately 548 million bushels were used to produce high fructose corn syrup for various food applications. Since *Bt* corn is grown on a significant percentage of corn acres planted in the US, there is a history of human consumption of food products derived from MON 810.

The Cry1Ab coding sequence (Hofte and Whitely, 1989) was isolated from the *B. thuringiensis* var. *kurstaki* (*Btk*) HD-1 strain present in DIPEL®,[2] a leading microbial insecticide in agricultural use. The United States Environmental Protection Agency (EPA) established an exemption from the requirement of a tolerance specifically for Cry1Ab protein in corn commodities (EPA, 1996). The conclusion of "reasonable certainty of no harm" and the resultant tolerance exemptions for this protein in food or feed is based on the lack of adverse effects in mammals in numerous toxicological studies. EPA has granted tolerance exemptions for many *Bt* Cry proteins as components of *Bt* microbial pesticides and as insect control proteins introduced into biotechnology-derived crops. The tolerance exemptions were based on the results of comprehensive toxicology testing which demonstrated the absence of adverse effects in non-target organisms and a history of safe use in agriculture for over 45 years (McClintock et al., 1995; EPA, 1998; Betz et al., 2000; Siegel, 2001; WHO, 1999; Federici, 2002).

The safety assessment includes a comparative assessment of the agronomic, compositional and feeding value of MON 810 with conventionally bred corn varieties (Dybing et al., 2002). The compositional comparison included numerous nutritional components for MON 810 and conventional corn grown in a variety of geographical conditions in the United States (1994) and in France (1995). The field trials and compositional analyses were carried out in compliance with EPA Good Laboratory Practice (GLP) standards. Grain was collected from replicated field trials and analyzed for proximates (e.g. protein, fat, ash), fiber, amino acids, fatty acids, and mineral content. Forage was also collected and analyzed for proximates and fiber. All comparisons showed that MON 810 is compositionally equivalent to conventional corn counterparts (Sanders et al., 1998).

Compositional equivalence studies confirm the absence of "unintended effects" in MON 810 when compared to conventional corn (Sanders et al., 1998). Animal feeding studies have also been undertaken to provide added confirmation of MON 810 safety and nutritional equivalence. These include feeding studies in ruminants, swine and poultry that demonstrate MON 810 supports the performance of farm animals comparable to that of animals fed conventional corn varieties. A 90-day feeding study was carried out in rats that provided confirmatory evidence of the safety of MON 810 for human consumption. The results of the 90-day "safety-assurance" study will be presented and be discussed in the context of the aforementioned studies to provide confirmatory evidence of the safety of MON 810 for human consumption.

2. Materials and methods

The study design was adapted from OECD Guideline No. 408 (1981) and was conducted in general compliance with OECD Good Laboratory Practice (GLP) guidelines at the Metabolism and Safety Evaluation – Newstead, toxicology laboratory, St. Louis, MO. The methods used were the same as those previously described in an earlier publication (Hammond et al., 2004b) and are briefly summarized below.

2.1. Test and control substances

The MON 810 and control grain (same background genetics as MON 810 but lacked the MON 810 coding sequence) were grown at the same time (1999) and in the same county in Colorado. The identity of MON 810 grain was confirmed by MON 810 event-specific polymerase chain reaction (PCR) analysis. The identity of the control variety was confirmed by the absence of the Cry1Ab protein using ELISA analysis. This study included the same reference grain used in a previous published study (Hammond et al., 2004b).

[1] YieldGard Cornborer is a registered trademark of Monsanto Technology, LLC.

[2] Dipel is a registered trademark of Valent Biosciences Corp.

2.2. Experimental diets

Diets containing test, control and reference grain were prepared in the same manner described previously (Hammond et al., 2004b). All diets were also tested for the presence of MON 810 grain using MON 810 event-specific PCR analysis to confirm the presence of the MON 810 event in diets containing the test grain and its absence in diets containing control and reference grain. Covance Laboratories (Madison, WI, USA) analyzed all formulated diets to determine if they met PMI specifications for 5002 diets.

2.3. Experimental design and treatment

This study was carried out at the same time and in the same animal room as a previously published study (Hammond et al., 2004b). The MON 810 study used the same source and age (6 weeks old at study start) of rats (Sprague Dawley derived Crl:CD® (SD) IGS BR), Charles River Laboratories (Raleigh, NC), followed the same experimental design and included the same reference groups. Table 1 contains an outline of the experimental groups and treatment regimen.

2.4. Clinical observations and pathology, statistical analysis

The same clinical observations, hematology, serum chemistry and urine chemistry measurements, organ weights, gross and microscopic pathology examinations and statistical procedures for data analysis described previously (Hammond et al., 2004b) were followed in this study.

3. Results

Compositional, contaminant, and nutritional content of the experimental diets met the specifications for Certified Rodent LabDiet 5002 established by PMI. The levels of heavy metals, aflatoxins, and chlorinated and organophosphate insecticides were below detection limits. For chlordane, the analytical limit of detection was higher (250 ppb) than the maximum allowable concentration of 50 ppb, but was not considered to have an impact on the study. PCR analysis confirmed that the test diet contained MON 810 as it tested positive for the Cry1Ab transformation event. The control and reference diets did not test positive for the Cry1Ab transformation event.

All of the 400 animals were healthy and appeared normal during the course of the study with the exception of one reference male that had to be sacrificed a few weeks prior to study termination due to injury. There were no changes noted during the duration of the study in behavior, activity, posture, gait, or external appearance that were considered to be test article related (data not shown).

Table 1
Experimental design

Group[a]	Animals/sex	State corn grown	Dietary level (% w/w)
1. Control	20	Colorado	11
2. Control	20	Colorado	33
3. MON 810	20	Colorado	11
4. MON 810	20	Colorado	33
5. Reference A	20	Ohio	33
6. Reference B	20	Iowa	33
7. Reference C	20	Indiana	33
8. Reference D	20	Ohio	33
9. Reference E	20	Colorado	33
10. Reference F	20	Colorado	33

[a] Control and reference grain are from conventional varieties that are not biotechnology-derived.

3.1. Body weight and food consumption

Overall, body weight and weight gain were comparable for MON 810, control and reference groups (Figs. 1 and 2). Food consumption was generally similar between MON 810, control, and reference groups throughout the course of the study (Figs. 3 and 4).

3.2. Clinical pathology parameters

Clinical pathology parameters (hematology, blood chemistry, urinalysis) were generally comparable for all groups. There were a few statistically significant differences between the MON 810 and the control groups after

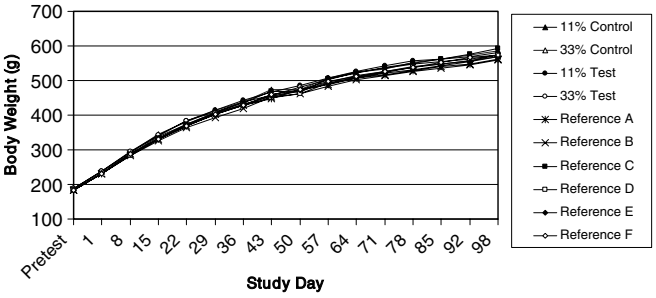

Fig. 1. Mean male body weights.

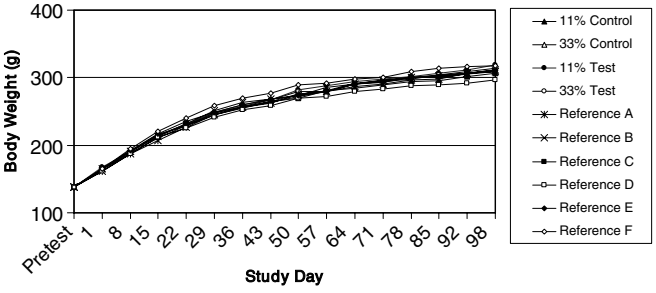

Fig. 2. Mean female body weights.

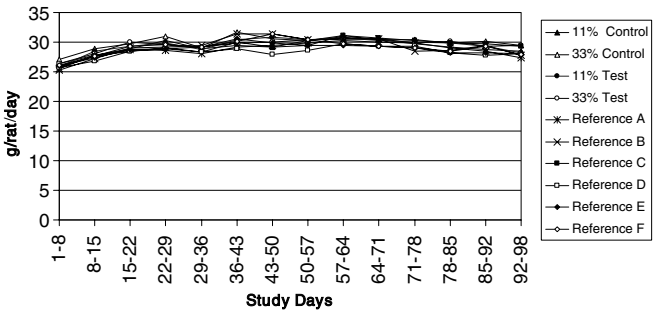

Fig. 3. Mean male food consumption.

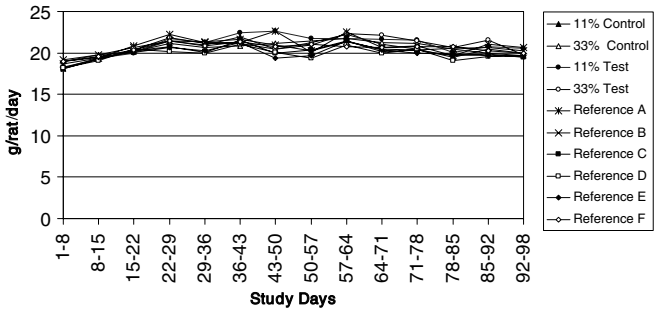

Fig. 4. Mean female food consumption.

4 weeks and at study termination. None of these differences were considered test article related since they were of small magnitude and within ±2SD of the mean for the population of reference groups, and were either different from the control or the reference group but not both, or were not dose related (observed at the low dose but not at the high dose), and/or occurred after 4 weeks but not at study termination.

3.2.1. Hematology

Results for males and females, measured at study termination (week 13), are contained in Tables 2 and 3, respectively. There were no statistically significant differences between male and female MON 810 groups and the controls and reference groups for most of the parameters measured. There were two statistically significant differences (MCHC, PLT) observed in females that were not dose related and therefore not considered to be test article related.

3.2.2. Serum chemistry

Results for males and females, measured at study termination (week 13), are contained in Tables 4 and 5, respectively. There were no statistically significant differences between male and female MON 810 groups and their control and reference groups for most of the parameters measured. The few statistically significant differences observed were not considered to be test article related as they were of small magnitude and fell within ±2SD of the mean of the reference groups (Hammond et al., 2004b). The slight reduction in A/G (albumin/globulin) ratio for high dose MON 810 males was attributed to the slightly lower albumin and slightly higher globulin levels for MON 810 males, neither of which were individually statistically different from controls. The A/G ratio for MON 810 males also fell within ±2SD of the mean

Table 2
Hematology mean values ± SD in male rats following 90 days of exposure to MON 810

Parameter	*N*	11% Control	33% Control	11% MON 810	33% MON 810
WBC (10^3/μl)	10	9.49 ± 1.75	9.97 ± 2.83	9.18 ± 2.25	9.65 ± 3.91
NEU (10^3/μl)	10	1.22 ± 0.34	1.35 ± 0.44	1.32 ± 0.49	1.33 ± 0.71
LYM (10^3/μl)	10	7.63 ± 1.41	7.95 ± 2.56	7.20 ± 2.26	7.65 ± 3.26
RBC (10^6/μl)	10	8.45 ± 0.40	8.78 ± 0.48	8.58 ± 0.33	8.55 ± 0.32
HGB (g/dl)	10	14.6 ± 0.67	15.0 ± 0.40	15.0 ± 0.46	14.9 ± 0.59
HCT (%)	10	43.5 ± 2.12	44.3 ± 1.12	43.9 ± 1.20	44.0 ± 1.40
MCV (fl)	10	51.4 ± 1.58	50.5 ± 2.44	51.2 ± 1.13	51.5 ± 1.43
MCH (pg)	10	17.2 ± 0.53	17.1 ± 0.80	17.5 ± 0.57	17.5 ± 0.43
MCHC (g/dl)	10	33.5 ± 0.41	33.8 ± 0.38	34.1 ± 0.71	33.9 ± 0.39
PLT (10^3/μl)	10	1034 ± 150	1005 ± 149	1006 ± 151	976 ± 128
PT (s)	10	12.0 ± 0.62	11.9 ± 0.54	12.0 ± 0.52	11.9 ± 0.46
APTT (s)	10	16.4 ± 1.10	16.0 ± 1.22	16.1 ± 1.38	16.1 ± 1.65

There were no statistically significant differences.

Table 3
Hematology mean values ± SD in female rats following 90 days of exposure to MON 810

Parameter	*N*	11% Control	33% Control	11% MON 810	33% MON 810
WBC (10^3/μl)	10	7.52 ± 1.86	7.65 ± 1.55	8.60 ± 2.27	7.42 ± 1.54
NEU (10^3/μl)	10	0.94 ± 0.39	0.98 ± 0.41	1.29 ± 0.87	0.82 ± 0.20
LYM (10^3/μl)	10	6.11 ± 1.76	6.13 ± 1.31	6.63 ± 1.07	6.10 ± 1.30
RBC (10^6/μl)	10	8.16 ± 0.42	8.03 ± 0.25	8.04 ± 0.55	8.02 ± 0.41
HGB (g/dl)	10	14.9 ± 0.63	14.9 ± 0.42	14.6 ± 1.16	14.9 ± 0.60
HCT (%)	10	43.3 ± 1.93	43.2 ± 1.25	43.5 ± 3.09	43.4 ± 1.64
MCV (fl)	10	53.0 ± 1.32	53.8 ± 1.03	54.2 ± 2.65	54.1 ± 1.30
MCH (pg)	10	18.3 ± 0.39	18.5 ± 0.30	18.2 ± 0.80	18.6 ± 0.36
MCHC (g/dl)	10	34.5 ± 0.37	34.4 ± 0.42	33.6 ± 0.81*	34.2 ± 0.41
PLT (10^3/μl)	10	927 ± 96	969 ± 77	1075 ± 213*	907 ± 93
PT (s)	10	11.1 ± 0.38	11.1 ± 0.38	10.9 ± 0.38	11.0 ± 0.35
APTT (s)	10	13.5 ± 1.99	13.8 ± 1.21	15.1 ± 1.72	14.1 ± 1.00

Statistically significant difference $^*P < 0.05$.

Table 4
Serum chemistry mean values ± SD in male rats following 90 days of exposure to MON 810

Parameter	*N*	11% Control	33% Control	11% MON 810	33% MON 810
ALP (U/L)	10	79.8 ± 15	83.3 ± 20	77.6 ± 15	71.3 ± 11
ALT (U/L)	10	39.3 ± 8.7	42.2 ±7.6	41.8±4.4	38.1 ± 4.5
AST (U/L)	10	77.2 ± 12	82.3 ± 14	83.6 ± 9	74.9 ± 8
GGT (U/U)	10	ND	ND	ND	ND
BUN (mg/dl)	10	15.7 ± 1.9	16.3 ± 3.1	15.9 ± 1.5	16.1 ± 1.8
CREA (mg/dl)	10	0.44 ± 0.05	0.46 ± 0.05	0.47 ± 0.05	0.45 ± 0.05[a]
TBIL (mg/dl)	2–3	ND	ND	0.20 ± 0.00	0.20 ± 0.00
TP (g/dl)	10	6.88 ± 0.27	6.86 ± 0.34	6.88 ± 0.27	6.67 ± 0.42
ALB (g/dl)	10	4.29 ± 0.22	4.44 ± 0.16	4.30 ± 0.26	4.15 ± 0.22
A/G	10	1.66 ± 0.14	1.85 ± 0.18	1.68 ± 0.18	1.66 ± 0.13**[,b]
GLOB (g/dl)	10	2.59 ± 0.16	2.42 ± 0.24	2.58 ± 0.19	2.52 ± 0.25
GLU (mg/dl)	10	212 ± 31	213 ± 21	210 ± 23	207 ± 20
Ca (mg/dl)	10	11.4 ± 0.44	11.4 ± 0.44	11.2 ± 0.31	11.2 ± 0.60
P (mg/dl)	10	10.3 ± 1.48	10.5 ± 1.34	10.1 ± 1.22	10.5 ± 0.67
Na (mmol/L)	10	148 ± 2	149 ± 3	148 ± 3	145 ± 5
Cl (mmol/L)	10	105 ± 1	106 ± 2	106 ± 2	103 ± 3*
K (mmol/L)	10	6.72 ± 1.0	6.88 ± 1.2	6.99 ± 1.2	7.27 ± 1.1

ND = not determined as the data were below the limit of detection.
Statistically significant differences $^{*}P < 0.05$, $^{**}P < 0.01$.
[a] Statistically significant difference from reference population mean only, $^{**}P < 0.01$ (refer to Hammond et al., 2004b for reference population means).
[b] Statistically different from both control and reference population means.

Table 5
Serum chemistry mean values ± SD in female rats following 90 days of exposure to MON 810

Parameter	*N*	11% Control	33% Control	11% MON 810	33% MON 810
ALP (U/L)	10	50.4 ± 13	50.5 ± 16	44.4 ± 12	43.7 ± 12
ALT (U/L)	10	45 ± 26	43 ± 13	37 ± 8	46 ± 11
AST (U/L)	10	88.00 ± 22.44	84.40 ± 8.67	85.30 ± 14.60	89.50 ± 14.39
GGT (U/U)	10	ND	ND	ND	ND
BUN (mg/dl)	10	16.9 ± 1.8	17.2 ± 2.3	17.3 ± 3.1	18.6 ± 2.6
CREA (mg/dl)	10	0.49 ± 0.03	0.51 ± 0.06	0.50 ± 0.05	0.53 ± 0.05[a]
TBIL (mg/dl)	6–8	0.22 ± 0.04	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
TP (g/dl)	10	7.21 ± 0.38	7.36 ± 0.44	7.34 ± 0.57	7.24 ± 0.54
ALB (g/dl)	10	5.10 ± 0.28	5.16 ± 0.26	4.98 ± 0.81	5.06 ± 0.47
A/G	10	2.43 ± 0.22	2.37 ± 0.24	2.17 ± 0.51	2.36 ± 0.36
GLOB (g/dl)	10	2.11 ± 0.19	2.20 ± 0.24	2.36 ± 0.36	2.18 ± 0.28
GLU (mg/dl)	10	173 ± 29	180 ± 31	182 ± 34	173 ± 22
Ca (mg/dl)	10	11.5 ± 0.30	11.5 ± 0.51	11.6 ± 0.48	11.2 ± 0.33[a]
P (mg/dl)	10	9.88 ± 1.23	9.65 ± 0.92	10.34 ± 1.12	9.84 ± 0.94
Na (mmol/L)	10	146 ± 2.1	148 ± 1.9	147 ± 3.5	146 ± 2.7[a]
Cl (mmol/L)	10	107 ± 1.6	107 ± 2.2	107 ± 2.8	106 ± 1.7
K (mmol/L)	10	7.90 ± 2.01	7.39 ± 0.82	7.67 ± 1.13	7.61 ± 1.32

ND = not determined as the data were below the limit of detection.
[a] Statistically significant difference from reference population mean only, $^{**}P < 0.01$.

(1.79 ± 0.34) of the reference groups (Hammond et al., 2004b) even though it was slightly, but statistically significantly lower that both the control and reference groups. There were no differences in A/G ratio for females. This finding was not considered to be test article related.

3.2.3. Urine chemistry

There were a few statistically significant differences, but these occurred only in the males at the interim sampling period and none occurred at study termination (data not presented). None of the differences were concluded to be test article related.

3.3. Organ weights

There were no statistically significant differences noted in absolute or relative organ weights between treated and control animals. Table 6 presents organ weight data relative to body weights (absolute organ weights and organ weight relative to brain weight data are not shown).

3.4. Pathology

At necropsy, no gross or microscopic lesions were observed that were considered to be test article related

Table 6
Organ/body weight mean values ± SD (%) in male and female rats following 90 days of exposure to MON 810

Parameter	*N*	11% Control	33% Control	11% MON 810	33% MON 810
Males					
Adrenals	20	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Brain	20	0.43 ± 0.05	0.42 ± 0.04	0.42 ± 0.04	0.42 ± 0.04
Heart	20	0.35 ± 0.03	0.34 ± 0.03	033 ± 0.03	0.35 ± 0.03
Kidney	20	0.80 ± 0.08	0.78 ± 0.05	0.77 ± 0.06	0.79 ± 0.05
Liver	20	2.97 ± 0.33	2.94 ± 0.28	2.95 ± 0.21	2.92 ± 0.22
Spleen	20	0.17 ± 0.03	0.17 ± 0.02	0.16 ± 0.02	0.16 ± 0.02
Testes	20	0.67 ± 0.08	0.67 ± 0.08	0.65 ± 0.07	0.69 ± 0.07
Females					
Adrenals	20	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00
Brain	20	0.72 ± 0.06	0.71 ± 0.07	0.70 ± 0.08	0.71 ± 0.07
Heart	20	0.38 ± 0.04	0.38 ± 0.04	0.39 ± 0.03	0.40 ± 0.04
Kidney	20	0.78 ± 0.06	0.77 ± 0.07	0.81 ± 0.06	0.77 ± 0.08
Liver	20	2.90 ± 0.28	2.90 ± 0.26	3.05 ± 0.37	2.98 ± 0.21
Spleen	20	0.19 ± 0.02	0.19 ± 0.03	0.21 ± 0.08	0.20 ± 0.05
Ovaries	20	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01

There were no statistically significant differences.

Table 7
Summary incidence microscopic findings in high dose (33%) male and female rats following 90 days of exposure to MON 810

Tissue	Microscopic finding	Control Males *N* = 20	MON 810 Males *N* = 20	Control Females *N* = 20	MON 810 Females *N* = 20
Heart	Cardiomyopathy	5	6	3	3
Kidney	Infiltrate, mononuclear cell	11	9	10	11
	Regeneration, tubular epithelium	16	20	3	5
	Tubular mineralization	1	2	6	7
	Cystic tubules	0	0	1	3
Liver	Infiltrate, mononuclear cell	9	10	5	7
	Inflammation, chronic, multifocal	19	17	17	18
Pancreas	Infiltrate, mononuclear cell	4	1	1	2
	Inflammation, chronic, focal	3	0	0	0
Thyroid	Cyst, ultimobranchial	1	1	4	4

There were no statistically significant differences.

(Table 7). The few spontaneous findings that were observed were generally of minimal to slight/mild severity, were randomly distributed among all groups and were the type commonly observed in control rats of this age and strain.

4. Discussion

During the course of this study, animals fed MON 810 in the diet had similar body weights, body weight gains, and food consumption when compared to animals fed diets containing grain from conventional varieties. In addition, there were no test article related differences in hematology, serum chemistry, and urinalysis parameters for MON 810 fed animals. There were no test article related changes in organ weights or gross and microscopic pathology. The summary prepared by the GMO Panel of the European Food Safety Authority also supports this conclusion. Following their review of the MON 810 rat feeding study, they concluded that the results of the study do not indicate adverse effects from consumption of maize line MON 810 (EFSA, 2004b).

The comparable responses of rats fed MON 810 to rats fed control grain supports the absence of untoward pleiotropic effects in MON 810 corn as confirmed in comprehensive composition studies as well as feeding studies in swine, poultry and ruminants with MON 810 and related *Bt* corn varieties that produce Cry1Ab protein in planta (Taylor et al., 2003; Weber et al., 2000; Donkin et al., 2003; Hammond et al., 2002).

Monitoring body weight gain and food consumption in feeding studies with rats, ruminants, pigs and poultry can be a sensitive indicator of overall animal health (Borzelleca, 1996). The absence of any meaningful differences in these measured parameters across a variety of animal species provides further support for the comparability of MON 810 to conventional corn varieties. A safety margin of approximately 75-fold was achieved when rat consumption of MON 810 grain (~21 g/kg/day) was compared to potential human dietary consumption (0.27 g/kg/day – 50th percentile) (DEEM^{TM}, 2002) even under the most conservative assumption that all corn products consumed were derived from MON 810. Since MON 810 is not grown on 100%

of the acres planted in the US, the safety margin would be higher than 75-fold.

The safety assessment of the introduced Cry1Ab protein has been previously addressed; the level of Cry1Ab protein in grain is approximately 0.3 ppm (Sanders et al., 1998). Mice administered an acute oral high dose of Cry1Ab protein (up to 4000 mg/kg) experienced no adverse effects. Thus, 4000 mg/kg of Cry1Ab protein was considered to be the no-effect level (NOEL). This dosage provides an approximate 50 million-fold safety factor when comparing potential human dietary exposure in the US (0.27 g/kg adult corn consumption × 0.3 μg Cry1Ab protein/g corn) to the mouse NOEL. An adult human would have to consume 933,333 kg of MON 810 corn in one day to achieve the same exposure to Cry1Ab protein that produced no adverse effects in mice. Since Cry proteins act through acute mechanisms to control targeted insect pests, high dose acute toxicity tests are considered appropriate to assess their potential effects on non-target organisms (Betz et al., 2000; Pariza and Johnson, 2001; Sjoblad et al., 1992). Based on the results the 90-day rat feeding study, the mouse acute study, and the farm animal feeding studies, the introduction of Cry1Ab protein into corn to provide protection against insect pests poses no meaningful safety risks.

5. Conclusion

The findings from rats fed diets containing MON 810 grain were similar to those fed diets containing grain from conventional control and reference varieties. The ''no-observed effect level'' is equal to the highest dietary level of MON 810 (33%) fed to rats. Substantial safety margins exist for human consumption of MON 810 grain based on the rat feeding study and are supported by a lack of adverse effects in numerous farm animal feeding studies. Consistent with agronomic, compositional and farm animal feeding studies, the 90-day rat feeding study did not detect unintended effects in the grain. Thus, MON 810 is considered to be substantially equivalent to, and as safe and nutritious as, conventional corn varieties.

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