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

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Abstract

The results of a 90-day rat feeding study with YieldGard® (YieldGard Rootworm Corn is a registered trademark of Monsanto Technology, LLC.) Rootworm corn (MON 863) grain that is protected against feeding damage caused by corn rootworm larvae are presented. Corn rootworm-protection was accomplished through the introduction of a *cry3Bb1* coding sequence into the corn genome for *in planta* production of a modified Cry3Bb1 protein from *Bacillus thuringiensis*. Grain from MON 863 and its near isogenic control were separately formulated into rodent diets at levels of 11% and 33% (w/w) by Purina Mills, Inc. Additionally, six groups of rats were fed diets containing grain from different conventional (non-biotechnology-derived) reference varieties. The responses of rats fed diets containing MON 863 were compared to those of rats fed grain from conventional corn varieties. All diets were nutritionally balanced and conformed to Purina Mills, Inc. specifications for Certified LabDiet 5002. There were a total of 400 rats in the study divided into 10 groups of 20 rats/sex/group. Overall health, body weight gain, food consumption, clinical pathology parameters (hematology, blood chemistry, urinalysis), organ weights, gross and microscopic appearance of tissues were comparable between groups fed diets containing MON 863 and conventional corn varieties. This study complements extensive agronomic, compositional and farm animal feeding studies with MON 863 grain, confirming that it is as safe and nutritious as existing conventional corn varieties.

Keywords: Corn: genetically modified; Corn rootworm-protected; 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,

Abbreviations: ANOVA, analysis of variance; APHIS, Animal and Plant Health Inspection Service; *B.t.*, *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; NPTII, neomycin phosphotransferase II; 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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2003) and the European Food Safety Agency (EFSA, 2004a) established a safety assessment process to assure that foods produced from these new 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 theoretically cause unintended (pleiotropic) effects. The risk assessment for a new 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.

Corn rootworm-protected corn event MON 863 (hereafter referred to as MON 863) marketed under the brand name YieldGard®[1] Rootworm corn was produced by insertion of DNA sequences that encode a modified *Bacillus thuringiensis* (subspecies *kumamotoensis*) Cry3Bb1 protein that is selectively toxic to Coleopteran species such as corn rootworm larvae (*Diabrotica* sp.). The genetic insert in MON 863 also contains the coding sequence for the selectable marker, neomycin phosphotransferase type II (NPTII) that is also produced in the plant. Corn varieties containing MON 863 are afforded a level of root protection from corn rootworm (CRW) larval feeding that is comparable or superior to that offered by currently available conventional chemical insecticides, thereby enabling the reduction in the use of these chemical insecticides.

The deduced amino acid sequence of the *B.t.* Cry protein (653 amino acids) produced in MON 863 is >98.9% identical to that of the Cry3Bb1 protein contained in the foliar-applied commercial *B.t.* microbial product, Raven® Oil Flowable Bioinsecticide (Ecogen, Inc.). The United States Environmental Protection Agency (EPA) recently established an exemption from the requirement of a tolerance specifically for Cry3Bb1 protein in corn commodities (EPA, 2004). 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 *B.t.* Cry proteins based on the results of extensive toxicity testing which show no adverse effects and a history of safe use in agriculture for over 45 years for *B.t.* microbial sprays (McClintock et al., 1995; EPA, 1998; Betz et al., 2000; Siegel, 2001; WHO, 1999; Federici, 2002).

The bacteria *Escherichia coli* was the source of the *nptII* gene isolated from prokaryotic transposon Tn5. The enzyme (264 amino acids) encoded by this gene (i.e. NPTII) is a widely used dominant selectable marker that was used in the development of MON 863. *E. coli* is ubiquitous in nature and found in the digestive tracts of vertebrate species, including humans (Jefferson et al., 1986). *E. coli* strains are commonly used as protein production systems in many commercial applications (Bogosian and Kane, 1991). Safety of the donor organism, *E. coli*, has previously been assessed by FDA as part of the consultation process for other transformed crops that contain the same *nptII* gene (FDA, 1998). In a recent publication from the European Network on Safety Assessment of Genetically Modified (GM) Food Crops (ENTRANSFOOD), the NPTII protein was assigned to group 1, antibiotic markers that pose the least risk for spread of antibiotic resistance genes in the environment (van den Eede et al., 2004). The authors acknowledged a 13 year history of safe use of the NPTII marker in agricultural food crops.

The second aspect of this safety assessment includes testing for potential pleiotropic effects resulting from insertion of the *cry3Bb1* and *nptII* coding sequence into the MON 863 genome. Testing involves a comparative safety assessment of MON 863 with conventionally bred corn varieties (Dybing et al., 2002). The comparative safety assessment includes three main components: (1) field assessments of key agronomic parameters, (2) compositional assessment of corn grain and forage, and (3) nutritional/safety assessment of MON 863 grain and forage fed to livestock.

Agronomic assessments for corn include examination of various parameters such as yield, plant height, silk date, dropped ears, stalk rating, root strength, plant vigor, and susceptibility to pathogens/pests, etc. (Astwood and Fuchs, 2000). These parameters have been measured by corn breeders following many years of conventional breeding of corn, and are used in selecting varieties that have optimal agronomic characteristics to ensure performance in the market place. Agronomic equivalency trials with multiple MON 863 varieties conducted at multiple locations demonstrate that MON 863 is agronomically equivalent to its near isogenic conventional counterparts except for the intentionally added corn rootworm-protected phenotype (Ward, 2001).

Compositional assessments included a comparison of 51 nutritional biochemical components of MON 863 to conventional corn grown during 1999 in the United States, and Argentina. 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 (protein, fat, ash, etc.), fiber, amino acids, fatty acids, vitamin E, mineral content, phytic acid, trypsin inhibitor, and selected secondary metabolites. Forage

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

was also collected and analyzed for proximates and fiber. All comparisons showed that MON 863 is compositionally equivalent to its near-isogenic conventional counterpart (George et al., 2004).

Agronomic and compositional equivalence studies confirm the absence of ''unintended effects'' in MON 863 when compared to conventional corn. Animal feeding studies have been undertaken to provide confirmation of MON 863 safety and nutritional equivalence. These include feeding studies in ruminants, swine and poultry that demonstrate MON 863 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 863 for human consumption. The results of the 90-day ''safety-assurance'' study will be presented and discussed in the context of the aforementioned studies to provide confirmatory evidence of the safety of MON 863 corn grain 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 Covance Laboratories, Vienna, Virginia, US.

2.1. Animals and maintenance

Male and female Sprague-Dawley derived rats (Crl:CD®(SD)IGS BR) from Charles River Laboratories (Kingston, NY) were approximately 6 weeks of age at study start. Rats were housed individually and provided food and water ad libitum. The testing facility provided appropriate environmental conditions for housing of animals (18–26 °C room temperature, 12-h light/dark cycle, 30–70% humidity, 10 air changes/h). Cage and rack location within the animal room was rotated every 7 days (±2 days).

2.2. Test and control substances

MON 863 and its near-isogenic control (i.e. a variety of corn of comparable genetics but lacks the *cry3Bb1* and *nptII* coding sequences), were grown under controlled conditions at isolated field sites in Hawaii during 2000 in compliance with USDA/APHIS guidelines for planting of regulated substances. Six conventional corn varieties representing a diversity of corn germplasm were also planted to serve as reference controls for the rat feeding study. Variables measured in rats fed these reference varieties can be considered to approximate the normal range of biological responses for the larger population of control rats. Grain harvested from these production fields served as test, control, and reference articles for the 90-day feeding study. Prior to the advent of biotechnology, newly developed corn varieties were not fed to rats in 90-day toxicology studies.

The grain samples used for diet preparation were analyzed for composition, pesticide residues (Covance Laboratories, Madison, WI) and mycotoxins (Romer Labs, Union, MO). The identity of the test article was confirmed by MON 863 event-specific polymerase chain reaction (PCR) analysis. The control grain was confirmed to be free of MON 863 by the same event-specific PCR test. The grain from five of the six conventional varieties used as reference controls in the MON 863 rat feeding study was produced in two Monsanto controlled field test sites, one in Illinois, and the other in Hawaii. The grain was produced in compliance with USDA/APHIS guidelines and in accordance with agronomic and cultural practices that are typical for corn production in the region they were grown. Field isolation practices met or exceeded USDA/APHIS guidelines to minimize pollen flow to corn grown inside or outside of the controlled field test sites. Test plots within a controlled field test site were separated by bare ground and/or temporal isolation. Test plots and containers holding the harvested grain were labeled and the harvesting equipment was cleaned between harvesting of each test plot. Grain from the conventional reference controls produced in the controlled field test sites was tested either by event-specific PCR, ELISA or lateral flow (gene-strip check) methods to verify they tested negative for genetically modified varieties also grown in the test sites (Roundup Ready corn at the Hawaii site, corn rootworm-protected corn at the Illinois site). One conventional reference variety was purchased from a contract research facility in Illinois and tested negative for MON 810 and GA21 (Roundup Ready) using event-specific PCR. During 1999, MON 810 and GA 21 were the predominant commercial biotechnology-derived varieties grown in the US on approximately 20% of planted corn acres. Finally, all of the rodent diets containing 33% control and reference grain tested negative for MON 863 using event-specific PCR.

2.3. Experimental diets

Diets containing test, control and reference grain were formulated by Purina TestDiet (Richmond, IN) to be nutritionally and compositionally comparable to PMI Certified Rodent LabDiet®[2] 5002. Many toxicology laboratories use this diet in rodent feeding studies. MON 863 (test), control and reference grain were

[2] PMI Certified LabDiet 5002 is a registered trademark of Purina Mills, Inc.

separately ground and incorporated into diets at levels of approximately 33% w/w, the standard incorporation rate for Certified Rodent Lab Diet 5002. MON 863 test and control grain were also incorporated into diets at 11% w/w to serve as a lower dose group to assess the dose–response of effects that might be observed at the 33% dietary level. Conventional corn grain supplied by Purina TestDiet was added at 22% w/w to the 11% w/w corn grain diets to bring the total grain content up to 33% w/w, consistent with other diets. Grain from the six reference varieties was added to diets at a level of 33% w/w. Following diet preparation, samples of all diets were analyzed (Covance Laboratories, Madison, WI) to confirm that the formulated diets met PMI specifications for Certified 5002 Rodent Diet.

2.4. Experimental design and treatment

Following acclimation to laboratory conditions, animals were assigned to 1 of 10 experimental groups (20 rats/sex/group) by stratified randomization so that group mean body weights did not differ significantly ($p \leqslant 0.05$) among treatment groups and so that the weight variation did not exceed ± 2 standard deviations of the mean weight for each group. Table 1 contains an outline of the experimental groups and treatment regimen.

2.5. Clinical observations

All animals were observed twice daily for mortality and moribundity and once daily for overt signs of toxicity; physical examinations were given weekly. Individual body weights were obtained twice prior to group allocation, again on the first day of treatment and weekly thereafter. Individual food consumption was determined weekly. Animals continued on test, control, or reference diets for a minimum of 90 days.

Table 1
Experimental design

Group[a]	Animals/sex	State corn grown	Dietary level (% w/w)
1. Control	20	Hawaii	11
2. Control	20	Hawaii	33
3. MON 863	20	Hawaii	11
4. MON 863	20	Hawaii	33
5. Reference A	20	Illinois	33
6. Reference B	20	Illinois	33
7. Reference C	20	Hawaii[b]	33
8. Reference D	20	Hawaii[b]	33
9. Reference E	20	Hawaii[b]	33
10. Reference F	20	Illinois	33

[a] Control and reference grain are from conventional varieties that are not biotechnology-derived.
[b] Grown in the same geographical location, but different from the locality where MON 863 and its control were grown.

2.6. Clinical pathology

Blood was collected via the jugular vein from the first 10 surviving rats/sex/group during week 5 and again at terminal sacrifice after week 13. Animals were fasted overnight (approximately 18–20 h) but did have access to water prior to blood collection. When possible, blood samples were collected from the same 10 animals at both collection periods.

2.6.1. Hematology

Hematology parameters (as cited in Tables 2 and 3) included red blood cell count (RBC), total leukocyte (WBC) and leukocyte differential count (NEU, LYM, etc.), platelet count (PLT), hematocrit (HCT), hemoglobin concentration (HGB), and red blood cell indices (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)). Whole blood was treated with anticoagulant (potassium EDTA), and hematology parameters were measured using a Technicon H1E™ System (Miles Inc., Elkhart, IN) blood cell counter. Plasma prothrombin time (PT) and activated partial thromboplastin time (APTT) were determined in an MLE Electra 1600C (Beckman Coulter, Miami, FL) from animals at terminal sacrifice using whole blood collected with sodium citrate as an anticoagulant.

2.6.2. Serum chemistry

Serum chemistry parameters (as cited in Tables 4 and 5) included albumin (ALB), globulin (GLB—calculated), total protein (TP), blood urea nitrogen (BUN), total bilirubin (TBIL), direct bilirubin (DBIL), glucose (GLU), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), creatinine (CREA), albumin/globulin ratio (A/G Ratio), cholesterol (CHOL), triglycerides (trig), calcium (CA), phosphorus (PHOS), chloride (CL), sodium (NA), and potassium (K). Serum chemistry parameters were measured using a Hitachi 912 clinical analyzer (Roche Diagnostics, Indianapolis, IN).

2.6.3. Urine chemistry

Urine was collected over ice for 18–20 h from the same rats used for blood collection. Protein, pH, blood, ketones, bilirubin, glucose, and urobilinogen were assayed in urine samples with MULTISTIX™ reagent-strips and a CLINI-TEK™ urinalysis strip reader (Ames Company, Elkhart, IN). Urine volume was measured and urine specific gravity was determined using an American Optical T.S. Meter. Urine appearance and opacity were determined by inspection and reported by exception. Sediment derived from centrifuging the urine sample was examined microscopically to determine the

Table 2
Hematology mean values ± SD in male rats following 90 days of exposure to MON 863 grain in the diet

Parameter	N	11% Control	33% Control	11% MON 863	33% MON 863	N	Reference population mean ± 2SD
WBC (10^3/μl)	10	7.27 ± 2.31	8.64 ± 2.24	8.39 ± 1.20	10.40 ± 1.57**	58	7.95 ± 3.82
NEU (10^3/μl)	10	0.96 ± 0.25	1.03 ± 0.27	1.16 ± 0.40	1.13 ± 0.19	58	1.14 ± 0.98
LYM (10^3/μl)	10	5.88 ± 2.10	7.21 ± 2.30	6.71 ± 1.11	8.80 ± 1.48**	58	6.41 ± 3.56
Baso (10^3/μl)	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.05**	58	0.01 ± 0.04
RBC (10^6/μl)	10	9.45 ± 0.39	9.44 ± 0.43	9.24 ± 0.39	9.35 ± 0.45	58	9.27 ± 1.05
HGB (g/dl)	10	16.6 ± 0.3	17.0 ± 0.5	16.4 ± 0.5	16.9 ± 0.3	58	16.7 ± 1.62
HCT (%)	10	46.9 ± 0.7	48.4 ± 1.4	46.2 ± 1.2	48.1 ± 0.9	58	47.7 ± 4.8
Retic count	10	0.06 ± 0.03	0.07 ± 0.04	0.07 ± 0.03	0.06 ± 0.04	58	0.06 ± 0.06
MCV (fl)	10	49.7 ± 1.64	51.3 ± 2.43	50.0 ± 1.71	51.4 ± 2.16	58	51.5 ± 4.8
MCH (pg)	10	17.6 ± 0.60	18.0 ± 0.73	17.7 ± 0.70	18.1 ± 0.73	58	18.1 ± 1.61
MCHC (g/dl)	10	35.4 ± 0.28	35.2 ± 0.50	35.5 ± 0.51	35.1 ± 0.60	58	35.0 ± 1.3
PLT (10^3/μl)	10	1041 ± 90	1103 ± 147	1057 ± 64	1139 ± 137	58	1065 ± 356
PT (s)	10	15.4 ± 0.47	15.3 ± 0.39	15.2 ± 0.51	15.3 ± 0.35	59	15.0 ± 1.46
APTT (s)	10	19.3 ± 1.22	19.8 ± 0.94	18.8 ± 1.82	20.0 ± 0.92[a]	58	21.8 ± 5.76

Statistically significant differences $^*P < 0.05$, $^{**}P < 0.01$.
[a] Statistically significant difference from reference population mean only, $P < 0.05$.

Table 3
Hematology mean values ± SD in female rats following 90 days of exposure to MON 863 grain in the diet

Parameter	N	11% Control	33% Control	11% MON 863	33% MON 863	N	Reference population mean ± 2SD
WBC (10^3/μl)	9–10	6.78 ± 1.71	5.64 ± 1.52	8.20 ± 1.59	6.78 ± 2.20	58	6.43 ± 3.56
NEU (10^3/μl)	9–10	0.80 ± 0.26	0.66 ± 0.24	0.83 ± 0.41	0.77 ± 0.25	58	0.65 ± 0.58
Baso (10^3/μl)	9–10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.03	58	0.00 ± 0.00
LYM (10^3/μl)	9–10	5.69 ± 1.52	4.73 ± 1.39	7.06 ± 1.32	5.74 ± 1.98	58	5.52 ± 3.28
RBC (10^6/μl)	9–10	8.64 ± 0.28	8.35 ± 0.63	8.65 ± 0.33	8.60 ± 0.18	58	8.53 ± 0.73
HGB (g/dl)	9–10	16.3 ± 0.3	16.1 ± 0.5	16.4 ± 0.5	16.3 ± 0.5	58	16.2 ± 1.07
HCT (%)	9–10	45.8 ± 1.1	45.6 ± 1.4	46.3 ± 2.0	45.8 ± 1.7	58	46.1 ± 3.6
Retic count	9–10	0.09 ± 0.04	0.09 ± 0.05	0.06 ± 0.04	0.04 ± 0.03	58	0.07 ± 0.08
MCV (fl)	9–10	53.1 ± 1.26	54.8 ± 3.88	53.5 ± 2.29	53.2 ± 1.93	58	54.1 ± 4.38
MCH (pg)	9–10	18.9 ± 0.45	19.4 ± 1.14	19.0 ± 0.74	18.9 ± 0.56	58	19.0 ± 1.34
MCHC (g/dl)	9–10	35.6 ± 0.23	35.4 ± 0.53	35.5 ± 0.46	35.6 ± 0.47	58	35.2 ± 0.96
PLT (10^3/μl)	9–10	1099 ± 288	1016 ± 140	1026 ± 337	991 ± 119	58	1047 ± 298
PT (s)	8–10	14.9 ± 0.42	15.3 ± 0.29	15.4 ± 0.20*	15.0 ± 0.45	56	14.7 ± 0.80
APTT (s)	8–10	17.0 ± 1.91	15.8 ± 1.57	17.2 ± 1.22	16.5 ± 1.10[a]	56	20.0 ± 4.80

Statistically significant differences $^*P < 0.05$.
[a] Statistically significant difference from reference population mean only, $P < 0.01$.

presence of bacteria, epithelial cells, erythrocytes, leukocytes, casts, or abnormal crystals. Urine sodium, potassium, chloride, calcium, creatinine and phosphorus concentration, as well as excretion (calculated), were determined quantitatively using a Hitachi 912 clinical analyzer.

2.7. Pathology

Following the 90-day exposure period to test, control and reference diets, all animals were anesthetized with sodium methohexital, sacrificed by exsanguination and given a complete gross pathologic examination. Adrenals, brain, heart, kidneys, liver, spleen, testes with epididymis, and ovaries were weighed; paired organs were weighed together. A full set of tissues was collected from all animals, including: aorta, adrenals, bladder, brain, cervix, epididymides, esophagus, eyes, femur with joint and marrow, heart, intestine (ileum, jejunum, duodenum, colon, caecum), kidneys, lacrimal gland, lesions or abnormal masses, liver, lungs (with mainstream bronchi), lymph node (mesenteric), ovaries, pancreas, peripheral nerve (sciatic), pituitary, prostate, rectum, salivary glands, seminal vesicles, skeletal muscle (thigh), skin (and mammary tissue in females), spinal cord (three levels), spleen, sternum with marrow, stomach, testes, thymus, thyroid/parathyroid, trachea, uterus and vagina. Following collection, tissues were placed directly into 10% neutral buffered formalin for fixation.

Adrenals, brain, colon, duodenum, heart, ileum, jejunum, kidneys, liver, lymph node (mesenteric), ovaries, pancreas, rectum, spleen, stomach, testes, and thyroid/parathyroid were examined histologically from all animals fed 33% MON 863 and 33% control grain. The microscopic examination was performed by a pathologist at Covance laboratories.

Table 4
Serum chemistry mean values ± SD in male rats following 90 days of exposure to MON 863 grain in the diet

Parameter	N	11% Control	33% Control	11% MON 863	33% MON 863	N	Reference population mean ± 2SD
ALP (U/l)	10	118.6 ± 25	106.3 ± 31	105.8 ± 20	107.5 ± 22[a]	60	91.5 ± 36
ALT (U/l)	10	67.1 ± 35.0	55.0 ± 17.5	47.3 ± 4.5	50.3 ± 8.3	60	51.7 ± 36.0
AST (U/l)	10	133.3 ± 94	99.2 ± 28	97.0 ± 15	94.4 ± 15	60	103.4 ± 70
GGT (U/l)	10	0.50 ± 0.71	0.20 ± 0.42	0.10 ± 0.32	0.20 ± 0.42	60	0.20 ± 0.88
BUN (mg/dl)	10	15.4 ± 1.4	14.0 ± 2.1	14.1 ± 1.4	14.7 ± 3.2	60	14.6 ± 0.76
CREA (mg/dl)	10	0.58 ± 0.04	0.52 ± 0.04	0.54 ± 0.05	0.59 ± 0.09	60	0.55 ± 0.14
TBIL (mg/dl)	10	0.11 ± 0.03	0.14 ± 0.07	0.11 ± 0.03	0.11 ± 0.03	60	0.11 ± 0.10
TP (g/dl)	10	7.14 ± 0.29	6.86 ± 0.28	6.81 ± 0.31	7.20 ± 0.32	60	7.12 ± 0.60
ALB (g/dl)	10	4.41 ± 0.16	4.27 ± 0.25	4.30 ± 0.21	4.40 ± 0.22	60	4.30 ± 0.42
CHOL (mg/dl)	10	60.1 ± 12.8	65.3 ± 8.1	63.9 ± 11.1	64.6 ± 12.2	60	61.8 ± 25.4
TRIG (mg/dl)	10	65.0 ± 18.8	69.8 ± 28.2	74.6 ± 27.0	70.6 ± 13.5	60	57.5 ± 43.6
A/G	10	1.63 ± 0.17	1.66 ± 0.20	1.73 ± 0.20	1.59 ± 0.23	60	1.55 ± 0.34
GLOB (g/dl)	10	2.73 ± 0.27	2.59 ± 0.21	2.51 ± 0.24	2.80 ± 0.32	60	2.81 ± 0.50
GLU (mg/dl)	10	108 ± 6	109 ± 6	105 ± 8	116 ± 20	60	110 ± 17
CA (mg/dl)	10	11.0 ± 0.64	10.7 ± 0.69	10.7 ± 0.45	11.3 ± 0.56	60	11.0 ± 1.24
PHOS (mg/dl)	10	6.99 ± 1.00	7.72 ± 0.84	7.19 ± 0.75	8.16 ± 0.70	60	7.6 ± 1.4
NA (mmol/l)	10	153 ± 2.5	152 ± 2.0	150 ± 0.9**	151 ± 2.9	60	153 ± 6.6
CL (mmol/l)	10	107.7 ± 2.7	107.5 ± 1.7	107.2 ± 1.4	104.8 ± 2.2*	60	105 ± 6.0
K (mmol/l)	10	5.70 ± 0.61	5.88 ± 0.45	5.59 ± 0.39	5.85 ± 0.73	60	5.88 ± 1.04

Statistically significant differences $^{*}P < 0.05$, $^{**}P < 0.01$.
[a] Statistically significant difference from reference population mean only, $P < 0.05$.

Table 5
Serum chemistry mean values ± SD in female rats following 90 days of exposure to MON 863 grain in the diet

Parameter	N	11% Control	33% Control	11% MON 863	33% MON 863	N	Reference population mean ± 2SD
ALP (U/l)	10	52.5 ± 16	52.5 ± 8	58.2 ± 17	48.8 ± 16	58	48 ± 22
ALT (U/l)	10	47.1 ± 16.1	41.0 ± 7.6	64.7 ± 50.8	39.4 ± 5.7	58	38.2 ± 27
AST (U/l)	10	96.5 ± 22	97.4 ± 9	149.4 ± 97	94.8 ± 12[a]	58	89 ± 56
GGT (U/l)	10	0.80 ± 0.63	0.80 ± 1.32	0.40 ± 0.70	0.60 ± 0.84	58	0.62 ± 1.18
BUN (mg/dl)	10	13.2 ± 2.3	14.6 ± 1.8	15.5 ± 2.5	14.4 ± 1.9	58	15.0 ± 3.48
CREA (mg/dl)	10	0.56 ± 0.05	0.61 ± 0.06	0.63 ± 0.07	0.60 ± 0.07	58	0.60 ± 0.12
TBIL (mg/dl)	10	0.18 ± 0.04	0.14 ± 0.05	0.18 ± 0.09	0.14 ± 0.05	58	0.14 ± 0.10
TP (g/dl)	10	7.35 ± 0.46	7.37 ± 0.51	7.39 ± 0.40	7.57 ± 0.46	58	7.60 ± 0.66
ALB (g/dl)	10	5.13 ± 0.33	4.86 ± 0.39	4.83 ± 0.29	5.11 ± 0.38	58	5.03 ± 0.64
CHOL (mg/dl)	10	74.9 ± 16.1	72.9 ± 13.2	81.9 ± 14.6	87.1 ± 24.6	58	84.5 ± 2.2
TRIG (mg/dl)	10	40.9 ± 12.3	43.9 ± 8.3	50.9 ± 7.8**	46.7 ± 15.0[a]	58	39.5 ± 15.2
A/G	10	2.33 ± 0.27	1.95 ± 0.21	1.91 ± 0.26**	2.09 ± 0.22	58	1.99 ± 0.52
GLOB (g/dl)	10	2.22 ± 0.25	2.51 ± 0.25	2.56 ± 0.31**	2.46 ± 0.21	58	2.57 ± 0.52
GLUC (mg/dl)	10	103 ± 8	105 ± 8	113 ± 11*	116 ± 8*	58	115 ± 22
CA (mg/dl)	10	11.1 ± 0.49	11.2 ± 0.33	11.1 ± 0.39	11.2 ± 0.32[a]	58	11.5 ± 1.3
PHOS (mg/dl)	10	6.79 ± 0.68	6.91 ± 1.33	7.18 ± 1.19	6.98 ± 1.16	58	6.30 ± 2.16
NA (mmol/l)	10	152 ± 2.4	150 ± 1.8	150 ± 2.5	152 ± 1.8	58	151 ± 5
CL (mmol/l)	10	107.8 ± 1.9	107.1 ± 2.0	107.0 ± 2.3	108.6 ± 2.2[b]	58	104 ± 4.8
K (mmol/l)	10	5.73 ± 0.54	5.72 ± 0.50	5.74 ± 0.48	5.62 ± 0.36	58	5.57 ± 1.04

Statistically significant differences $^{*}P < 0.05$, $^{**}P < 0.01$.
[a] Statistically significant difference from reference population mean only, $P < 0.05$.
[b] Statistically significant difference from reference population mean only, $P < 0.01$.

2.8. Statistical analysis

For quantitative measures, the 33% MON 863 group was compared to the 33% control group and the mean of the reference group population. The 11% MON 863 group was compared to the 11% control group. For each sex, a simple one-way analysis of variance (ANOVA) model was used to fit the data and specific treatment combinations were compared using contrasts. For the mean of the reference group population (mean of all individual animal values for reference groups A–F, Table 1), the comparisons were done using a one-degree of freedom *t*-test generated from a contrast between the MON 863 group (test) and either the control group or the population mean for the reference groups. Differences were considered statistically significant when both

the ANOVA and the contrast were statistically significant ($p \leqslant 0.05$). As part of the overall analysis, Levene's test was used to compare group variances. If Levene's test was significant ($p \leqslant 0.01$), the data were ranked and the analysis of ranked data was performed. The incidences of microscopic findings were analyzed using Fisher's Exact Test. SAS® version 8.2 (SAS Institute Inc., Cary, NC) was used for all statistical computations. For qualitative measures, the MON 863 (test) was compared to the control group using appropriate categorical analysis techniques.

3. Results

Compositional, contaminant, and nutritional analysis of the experimental diets showed that they met the specifications for Certified Rodent LabDiet 5002 established by PMI. The levels of heavy metals, aflatoxins, chlorinated hydrocarbons, and organophosphate insecticides were below detection limits. For chlordane, the Covance Laboratories' limit of detection was higher (250 ppb) than the maximum specified concentration of 50 ppb, but this was not considered to have an impact on the study. PCR analysis confirmed that the test diet contained MON 863. The control and reference diets tested negative for the presence of MON 863.

All of the 400 experimental animals were healthy and appeared normal during the course of the study. There were a few incidental deaths that were not considered related to treatment. One male from the 33% MON 863 group was found dead on day 92; the cause of death could not be determined as there were no unusual findings noted at macroscopic or microscopic examination of tissues. A control 33% male was sacrificed in extremis on day 64, a broken maxilla was found at necropsy. A reference group male was found dead on day 88, the cause of death was not apparent. Two reference group females died shortly after the interim blood collections at week 5. Their deaths were attributed to the blood collection procedure. There were no changes noted in behavior, activity, posture, gait, or external appearance in any of the groups in either sex throughout the course of the study (data not shown).

3.1. Body weight and food consumption

Overall, body weight and weight gain were comparable for the MON 863, control and reference groups (Fig. 1). A couple of statistically significant differences (increase at week 3, decrease at week 4) in cumulative weight gain between the 33% MON 863 and reference females were observed, but these differences were of small magnitude, were in opposite directions and there was no corresponding differences from female controls. As a consequence, these differences were not considered to be test article related.

Food consumption was generally similar between test, control and reference groups (Fig. 2). The slight reduction in food consumption in all groups (week 5) is attributed to the interim blood collection during that week which required overnight fasting of animals. There were two statistically significant differences observed between 11% MON 863 and control males (decreases at weeks 3 and 10), but there were no corresponding differences at the 33% dietary level. Since these findings were not dose-related, were of small magnitude, and did not appear consistently in the study, they were not considered to be test article related.

3.2. Clinical pathology parameters

Clinical pathology parameters were generally comparable for all groups. There were a few statistically significant differences observed between the MON 863 and control or reference groups after 5 weeks. These differences were not considered to be test article related since they were within ±2 standard deviations of the population mean for reference groups, and were either different from the references but not the control, or were not dose-related and/or were observed at the interim bleed but not at study termination. Results from the analyses at study termination (week 13) are presented as follows.

3.2.1. Hematology

Results for males and females measured at study termination (week 13) are contained in Tables 2 and 3, respectively. Statistically significant differences between the 33% MON 863 and the control and reference group mean were observed for a few parameters; male white blood cell count (WBC), male lymphocyte count and male absolute basophils. The slight increase in male 33% MON 863 WBC was largely due to the slight increase in lymphocyte count. All of the individual male WBC values in the 33% MON 863 group (8.4–13.1) were within the range of the individual animal reference control values (4.3–13.0) with the exception of one MON 863 male (value of 13.1). Similarly, all of the individual male 33% MON 863 lymphocyte counts (7.0–11.3) were within the range of values (3.5–11.4) for the reference male population. There were no differences in WBC count in MON 863 females. There were no corroborative histologic changes in the spleen and lymph nodes of MON 863 animals as they appeared within normal limits when examined microscopically.

3.2.2. Serum chemistry

Results for males and females from study termination (week 13) are contained in Tables 4 and 5 respectively. Statistically significant differences between the 33% MON 863 and control group were limited to a slight

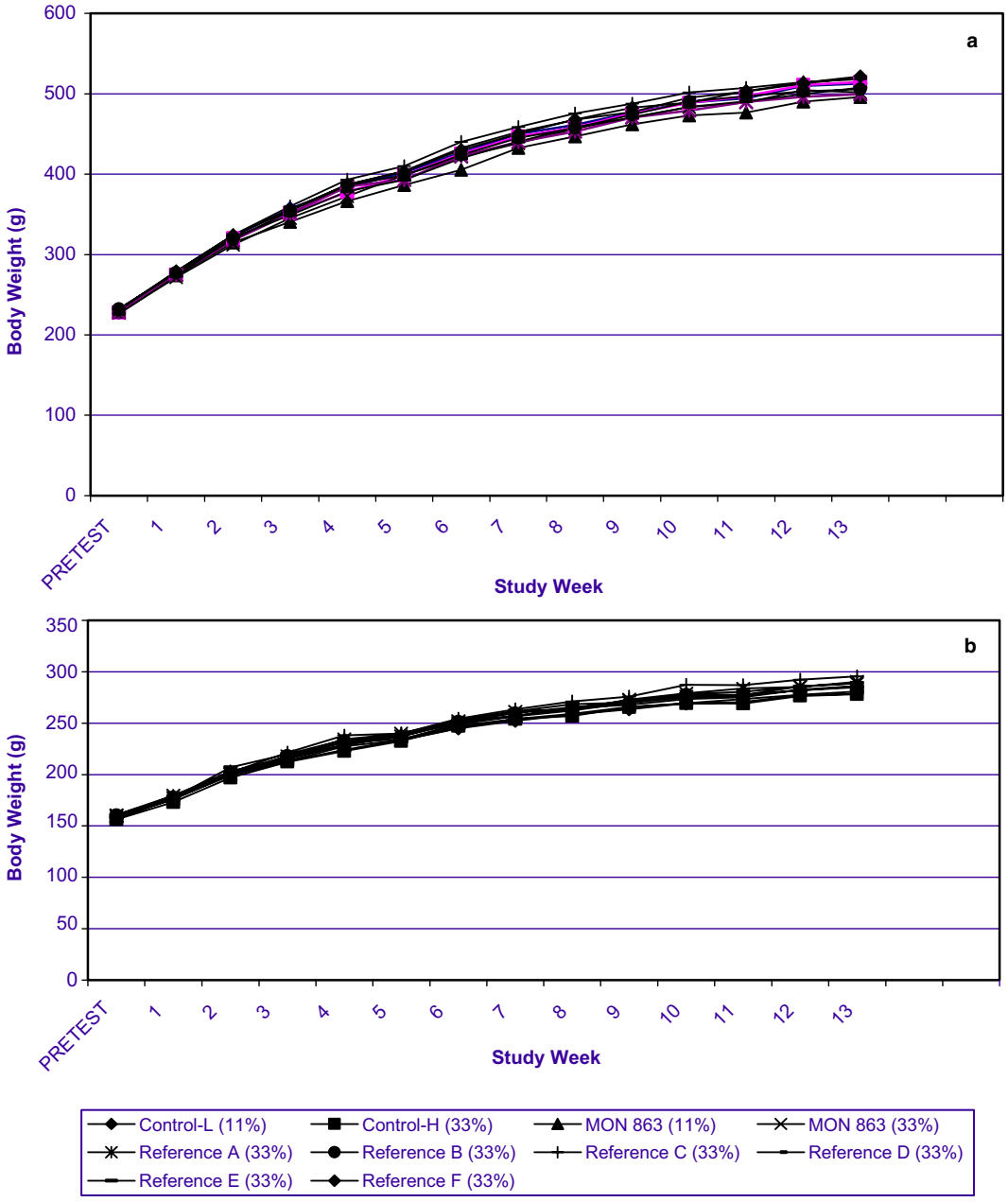

Fig. 1. Mean (a) male and (b) female body weights.

increase in glucose (MON 863 females only) and decrease in chloride (MON 863 males only). The range of individual animal glucose values (mg/dl) for MON 863 females was similar to the individual control animal range (11% MON 863: 94–133 compared to 11% controls: 88–115) and (33% MON 863: 103–126 compared to 33% controls: 97–122). The female 33% MON 863 individual glucose levels were also within the range of individual animal values (93–143) for female reference groups. Furthermore, the 11% and 33% female MON 863 mean glucose values (113 and 116 respectively) were less than the mean values for two of the reference groups: A (120) and D (117). Thus, the small change in glucose levels in MON 863 females was not considered to be test article related.

The slight decrease in male 33% MON 863 serum chloride (105) relative to the control (107) was attributed to the slightly elevated control chloride value relative to the other reference groups (104–106). As a consequence, serum chloride for 33% MON 863 males was not different from the population mean for reference groups. There were no statistical differences in serum chloride for females. All of the aforementioned statistical differences in serum chemistry were of small magnitude and fell within ±2 standard deviations of the reference population mean and were considered to be incidental

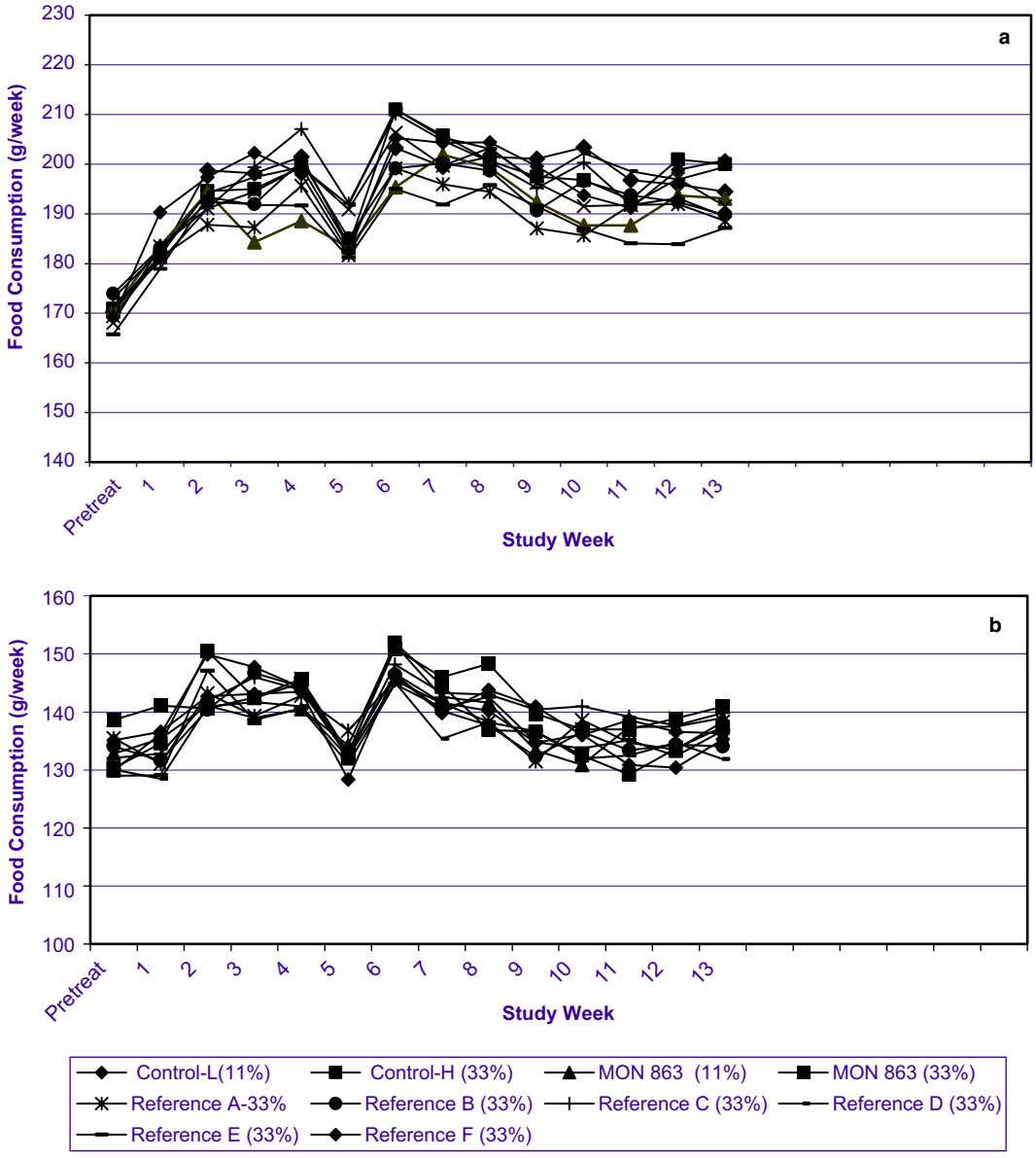

Fig. 2. Mean (a) male and (b) female food consumption.

findings and not related to administration of the test article.

3.2.3. Urine chemistry

There were no statistically significant differences in urinalysis parameters between the male and female 33% MON 863 group and the 33% control group (data not shown). Low dose MON 863 male urine specific gravity was slightly lower than low dose control male values, but this was not dose-related as there were no differences at the high dose. Urine sodium excretion for 33% MON 863 males was slightly lower than the reference population mean, but was not different from the 33% control group. These slight differences were not considered to be test article related.

3.3. Organ weights

There were no statistically significant differences between the 33% MON 863 and control groups in absolute organ weight (data not shown), organ weights as a percentage of body weight (Table 6) or organ weights as a percentage of brain weight (data not shown). The kidney/body weight ratio was slightly lower for MON 863 males (0.67) compared to controls (0.71), but this slight decrease was not statistically significant. In addition, the kidney/body weight ratio was very similar or identical to three of the male reference groups, A (0.68), D (0.67), F (0.67). Female MON 863, control and reference group absolute and relative kidney weights were similar.

Table 6
Organ/body weight mean values ± SD in male and female rats following 90 days of exposure to MON 863 grain in the diet

Parameter	*N*	11% Control	33% Control	11% MON 863	33% MON 863	*N*	Reference population mean ± 2SD
Males							
Adrenals	19–20	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	119	0.01 ± 0.00
Brain	19–20	0.45 ± 0.03	0.44 ± 0.05	0.47 ± 0.04	0.45 ± 0.03	119	0.44 ± 0.07
Heart	19–20	0.33 ± 0.03	0.33 ± 0.03	0.34 ± 0.04	0.34 ± 0.04	119	0.34 ± 0.08
Kidney	19–20	0.69 ± 0.05	0.71 ± 0.07	0.68 ± 0.05	0.67 ± 0.04	119	0.68 ± 0.13
Liver	19–20	2.57 ± 0.16	2.59 ± 0.19	2.62 ± 0.21	2.59 ± 0.18	119	2.61 ± 0.54
Spleen	19–20	0.15 ± 0.02	0.16 ± 0.01	0.16 ± 0.02	0.16 ± 0.02	119	0.16 ± 0.04
Testes	19–20	1.15 ± 0.10	1.11 ± 0.21	1.19 ± 0.11	1.15 ± 0.10	119	1.14 ± 0.24
Females							
Adrenals	20	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	118	0.03 ± 0.01
Brain	20	0.77 ± 0.06	0.76 ± 0.06	0.75 ± 0.05	0.74 ± 0.08	118	0.74 ± 0.12
Heart	20	0.39 ± 0.04	0.37 ± 0.02	0.37 ± 0.04	0.39 ± 0.03	118	0.38 ± 0.07
Kidney	20	0.76 ± 0.06	0.74 ± 0.06	0.75 ± 0.05	0.73 ± 0.08	118	0.73 ± 0.12
Liver	20	2.83 ± 0.19	2.91 ± 0.41	2.92 ± 0.20	2.98 ± 0.42	118	2.89 ± 0.67
Spleen	20	0.20 ± 0.03	0.19 ± 0.02	0.20 ± 0.03	0.19 ± 0.03	118	0.20 ± 0.06
Ovaries	20	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	118	0.06 ± 0.02

There were no statistically significant differences.

3.4. Pathology results

At necropsy, no gross lesions were observed that were considered to be test article related. The findings observed were randomly distributed among all groups and were the types commonly observed in rats of this age and strain. The incidence of microscopic changes observed in the tissues of the 33% MON 863 and control groups is shown in Table 7. The only statistically significant difference was a decreased incidence of kidney tubule mineralization in 33% MON 863 females compared to the control, a finding which is not considered to be adverse. There were small increases in the incidences of focal inflammation and tubular regenerative changes in the kidneys of 33% MON 863 males when compared to their controls. In contrast, the incidence of cardiomyopathy was decreased in 33% MON 863 males when compared to controls. These findings illustrate the variability in the incidence of common microscopic changes that occur in rats of this age and strain. Most of the microscopic findings were of minimal severity and none of the aforementioned pathology findings were

Table 7
Summary incidence microscopic findings in high dose (33%) male and female rats following 90 days of exposure to MON 863 grain in the diet

Tissue	Microscopic finding	Males		Females	
		Control, $N = 20$	MON 863, $N = 20$	Control, $N = 20$	MON 863, $N = 20$
Adrenal, cortex	Vacuolization	20	20	15	15
Heart	Cardiomyopathy	11	6	7	7
Kidney	Focal chronic inflammation	7	11	7	6
	Focal tubular regeneration	8	14	2	3
	Tubular mineralization	0	0	9	2*
Liver	Vacuolization	17	20	18	20
	Congestion	1	1	3	3
	Foci of chronic inflammation	17	17	19	18
	Bile duct, inflammation, chronic	6	10	5	6
	Bile duct hyperplasia	6	5	2	2
	Hemorrhage	0	2	2	0
	Necrosis (minimal)	0	3	1	0
Rectum	Parasitism	1	3	6	2
Spleen	Pigment, increased	18	20	19	20
Stomach	Dilation, glandular	1	4	1	2
Thyroid	Cyst, ultimobranchial	7	8	5	9

Statistically significant difference $^{*}P < 0.05$.

considered by the study pathologist to be test article related.

In response to questions from one regulatory reviewer in Europe, kidney tissues were subjected to an independent pathology peer review. Two independent board certified pathology experts carried out a blinded, pathology peer review of MON 863 and control kidney slides (both males and females) and reviewed relevant clinical pathology data and kidney weights. They concluded that the microscopic kidney changes observed in test and control animals represented classic chronic progressive nephropathy (CPN), a very common finding in rats, particularly males (Hard and Khan, 2004). The severity of CPN was generally minimal for MON 863 and control groups and the incidences were similar, 18/20 and 14/20 for MON 863 and control males, and 4/20 and 9/20 for MON 863 and control females, respectively. They concluded that there was no evidence of treatment related microscopic changes in the kidney. The slight decrease in male kidney weights was considered to be within normal limits, and the relevant clinical data reflected the absence of any treatment related kidney changes. Therefore, it was concluded that the kidneys of rats fed diets containing MON 863 were normal and comparable in appearance and function to kidneys from animals fed diets containing control grain.

4. Discussion

During the course of this study, animals fed MON 863 grain in the diet had similar body weights, body weight gains and food consumption when compared to animals fed diets containing grain from control or six different conventional reference varieties. In addition, there were no differences in hematology, serum chemistry, and urinalysis parameters for animals fed MON 863 that were considered test article related. Similarly, there were no test article related changes in organ weights or gross and microscopic pathology.

The comparable responses of rats fed MON 863 grain to those of rats fed control and reference grain supports the absence of unintended changes in MON 863 as determined in comprehensive agronomic and composition studies as well as confirmatory feeding studies in poultry, swine, dairy and beef cattle (Taylor et al., 2003; Fischer et al., 2003; Bressner et al., 2003; Grant et al., 2003; Wilson et al., 2003).

Body weight gain measured in the rat, swine, beef cattle and poultry feeding studies is a sensitive indicator of toxicity. In a comprehensive review of the relationship of chemical structure to toxicity for over 600 chemicals of divergent structure and toxicity, "no-observed-effect" levels (NOELs) were more frequently based on body weight changes than other clinical endpoints measured in subchronic and chronic rodent toxicology studies (Munro et al., 1996). Thus, monitoring body weight changes and food consumption in feeding studies with four different animal species fed large amounts of corn in the diet improves the sensitivity to detect unintended effects. As stated in a review of the safety assessment of macronutrients, which has some relevance to whole food safety assessment, "the single most effective way to evaluate the overall health status of an animal is to observe the effects of treatment on body weight, food consumption, and food efficiency" (Borzelleca, 1996).

It has been recognized that whole foods cannot be fed to laboratory animals at the high exposure levels used in the typical hazard assessment studies conducted with conventional pesticide chemicals and food additives (FAO, 1996; Dybing et al., 2002; Hammond et al., 1996). Typically, safety margins (animal exposure/human exposure) of at least 100-fold or greater are achieved in hazard assessment studies with chemicals. However, safety margins of less than 100-fold are often derived from studies with whole foods since there are limits to how much food laboratory animals can tolerate before nutritional problems intervene (Borzelleca, 1996). Attempts to achieve higher safety margins by feeding laboratory animals the whole food exclusively in the diet, and ignoring the nutritional consequences, can result in the generation of uninterpretable data. This was demonstrated years ago in some of the many toxicology studies carried out with irradiated foods. Feeding nutritionally unbalanced diets had negative effects on animal health that confounded interpretation of the study results (Pauli and Takeguchi, 1986). Furthermore, some foods that are wholesome for humans are not well tolerated when fed at exaggerated doses to laboratory animals (Elias, 1980; Hammond et al., 1996).

In the current study, MON 863 grain was formulated in rodent diets to avoid the limitations discussed above. The technical expertise of Purina Mills, Inc. was utilized to prepare nutritionally balanced diets for the laboratory rat that were formulated to meet the specifications of Certified Rodent LabDiet 5002. This diet is used in many toxicology research laboratories.

The dietary exposure of rats to MON 863 grain averaged over the 90-day study was approximately 21 g/kg body weight/day, which considerably exceeds human dietary exposures. Corn grain consumption in the aforementioned poultry and swine studies was approximately 57 and 26 g/kg body weight/day, respectively. United States mean adult per capita consumption of corn grain (endosperm fraction) is estimated to be approximately 0.27 g/kg/day using the Dietary Exposure Estimation Model (DEEM™, Exponent, Inc.) based on data from the USDA Continuing Survey of Food Intake for Individuals (1994–1996). This represents a safety margin of approximately 78-fold when rat dietary exposure is compared to that of humans assuming they consumed only MON 863 corn grain, a very conservative assumption.

In the animal feeding studies, 100% of the grain fed was from MON 863 corn. However, MON 863 corn will never represent 100% of the total corn grown in the United States for animal and human food. In the year 2000, there were approximately 80 million acres of corn planted, and approximately 14 million (18%) of these acres were treated with insecticides to control corn rootworm (Doane, 2001). Assuming 100% market penetration of those acres currently treated with corn rootworm insecticides, only about 18% of the total corn acres planted in the US would be MON 863, increasing the safety margin derived from feeding studies by a factor of 5 to approximately 432-fold (5×78-fold safety margin). In Europe, the safety margin would be even higher since MON 863 grain is not currently grown there, and any imported into Europe would be used primarily for animal feed (mostly corn gluten feed). The level of corn grain imported into the European Union (EU) is limited by the General Agreement on Tarriffs and Trades to only about 7% of the total corn grown in the EU. Less than 8% of the imported grain is used in human foods (Brookes, 2001). Thus, MON 863 imported into Europe would constitute a negligible fraction of the total amount of corn grain that enters the EU human food supply.

In regards to the safety assessment of the introduced proteins, Cry3Bb1 and NPTII both are present at low levels in MON 863. The mean Cry3Bb1 level in grain is 70 μg/g fresh wt. (range 49–86); for NPTII, it is below the limit of detection for the assay in grain, <0.076 μg/g fresh wt. (Monsanto, 2003). The safety of NPTII has been addressed in multiple publications (Fuchs et al., 1993; Flavel et al., 1992). The *nptII* gene is present in a number of commercial biotechnology-derived crops developed by Monsanto that have successfully completed the consultation process with FDA (2005). Lastly, the EPA has established an exemption from the requirement of a tolerance for NPTII and the genetic material necessary for its expression in or on raw agricultural commodities (EPA, 1994). Collectively, these regulatory actions confirm the safety of the NPTII protein.

The Cry3Bb1 protein is readily digested in simulated gastric fluid, similar to what has been observed with other Cry proteins (Betz et al., 2000; Leach et al., 2001; Hileman et al., 2001). It is not structurally related to known protein allergens or mammalian toxins based on bioinformatics searches of genetic data-bases such as Gen-Bank, EMBL, PIR and SwissProt (Leach et al., 2001; Hileman et al., 2001). The absence of toxicity of the Cry3Bb1 protein was confirmed in an acute mouse gavage study that was conducted to support an EPA tolerance exemption (Monsanto, 2003). Acute toxicity studies are considered to be appropriate to identify the potential toxicity of proteins such as Cry proteins since they act through acute mechanisms to control targeted insect pests (Sjoblad et al., 1992; Betz et al., 2000). No adverse effects were observed in mice given dosages up to 3200 mg/kg of Cry3Bb1 protein (Monsanto, 2003), consistent with the absence of toxicity observed with other Cry proteins fed to mammals. When the no-effect high dose of 3200 mg/kg for the Cry3Bb1 protein is compared to potential mean (50th percentile) human dietary exposure in the United States (0.27 g/kg/day × 0.07 mg/g) of MON 863 grain, a safety margin of approximately 170,000 is determined. This calculation is based on the very conservative assumption that no Cry3Bb1 protein is lost during processing of grain into human food, and that 100% of the daily dietary intake of corn grain is derived from MON 863. As discussed earlier, this safety margin would be increased approximately 5-fold given the projected market penetration of MON 863 in the US.

5. Conclusion

In conclusion, the findings from rats fed diets containing MON 863 grain were similar to those fed diets containing grain from control and conventional reference varieties. The results of this 90-day rat study have been subsequently reviewed in their entirety by multiple global regulatory agencies. The summary prepared by the GMO Panel of the European Food Safety Authority best captures the prevailing scientific conclusion regarding the findings from this study. EFSA concluded that the results of the 90-day rodent study do not indicate adverse effects from consumption of maize line MON 863 (EFSA, 2004b). The "no-observed effect level" is equal to the highest dietary level of MON 863 (33%) fed to rats. Substantial safety margins exist for human consumption of MON 863 grain in the US and in Europe based on the rat and farm animal feeding studies. Consistent with the other comprehensive studies that have been completed, the 90-day rat feeding study did not detect unintended effects in the grain. Thus, MON 863 is considered to be substantially equivalent to, and as safe and nutritious as, conventional corn varieties.

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