Food and Chemical Toxicology 46 (2008) 2517–2524

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Results of a 13-week safety assurance study with rats fed grain from corn rootworm-protected, glyphosate-tolerant MON 88017 corn

C. Healy [a,*], B. Hammond [a], J. Kirkpatrick [b]

[a] Monsanto Company, 800 North Lindbergh Blvd., St. Louis, MO 63167, United States
[b] WIL Research Laboratories, LLC, 1407 George Road, Ashland, OH 44805-9281, United States

ARTICLE INFO

Article history:
Received 23 August 2007
Accepted 8 April 2008

Keywords:
Corn: genetically modified
Corn: rootworm-protected
Rat feeding study

ABSTRACT

Presented are the results of a 13-week rat feeding study with grain from MON 88017 corn (brand name YieldGard VT Rootworm/RR2™), protected from feeding damage caused by corn rootworm and tolerant to glyphosate, the active ingredient in Roundup® agricultural herbicides. Corn rootworm protection is accomplished through the introduction of *cryBb1* coding sequence from *Bacillus thuringiensis* into the corn genome for *in planta* production of a bioactive form of Cry3Bb1 protein. Also included in the genome is the coding sequence for the CP4 EPSPS protein from *Agrobacterium* sp. strain CP4 that confers glyphosate herbicidal tolerance. MON 88017 was formulated into rodent diets at 11 or 33% (w/w) levels with its near isogenic control at a level of 33% (w/w). Additionally, six diets containing grain from different conventional (non-biotechnology-derived), reference hybrids were formulated, each at 33% (w/w) levels of one of six reference grains. All diets were nutritionally balanced and conformed to PMI specifications for Certified LabDiet® 5002 (PMI Certified LabDiet 5002 is a registered trademark of Purina Mills, Inc.). The responses of rats fed diets containing MON 88017 were comparable to those of rats fed a diet containing grain from its near isogenic control. This study complements extensive agronomic, compositional, and farm animal feeding studies with MON 88017 grain, confirming that it is as safe and nutritious as grain from existing commercial corn hybrids.

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 a "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, 1997a), the Codex Alimentarius Commission (Codex, 2003a), and the European Food Safety Agency (EFSA, 2004) 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 could theoretically cause unintended 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 (IFBC, 1990; Kessler et al., 1992; FAO/WHO, 2000; CAST, 2001; Chesson, 2001; Kuiper et al., 2001; Cockburn, 2002; Codex, 2003b). The outcome of this assessment is to determine whether the biotech crop is comparable to the existing conventionally bred crop (Dybing et al, 2002). Newly introduced proteins are also subject to a comprehensive safety evaluation.

MON 88017 corn grain[1] was produced by *Agrobacterium*-mediated insertion of DNA sequences on the same vector plasmid that encode (1) a modified *Bacillus thuringiensis* (subspecies *kumamotoensis*) Cry3Bb1 protein (Hammond et al., 2006a) that is selectively toxic to Coleopteran species such as corn rootworm larvae (*Diabrotica* sp.) and (2) CP4 EPSPS (5-enolpyruvylshikimate-3-phosphate synthase

Abbreviations: ANOVA, analysis of variance; CPN, chronic progressive nephropathy; CRW, corn rootworm; dwt, dry weight; EFSA, European Food Safety Agency; EPA, Environmental Protection Agency; FAO, Food and Agricultural Organization; GLP, Good Laboratory Practices; NOEL, no-effect level; OECD, Organization For Economic Cooperation and Development; PCR, polymerase chain reaction; PMI, Purina mills Inc.; SD, standard deviation; WHO, World Health Organization; US, United States; w/w, weight/weight.

* Corresponding author. Tel.: +1 314 694 8859; fax: +1 314 694 5925.
E-mail address: charles.e.healy@monsanto.com (C. Healy).

[1] Marketed under the brand name YieldGard VT Rootworm/RR2™, a trademark of Monsanto Technology LLC.

0278-6915/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2008.04.005

protein) that provides tolerance to glyphosate, the active ingredient in Roundup[2] agricultural herbicides. As described in Hammond et al. (2006a), the modification of the amino acid sequence of the Cry3Bb1 protein was minor, being 98.9% identical to that in the commercial *Bt* microbial product Raven® Oil Flowable Bioinsecticide (Ecogen, Inc.). The CryBb1 proteins have similar insecticidal potency against Coleopteran species and are non-toxic to mammals based on the results of high-dose acute toxicity tests. The Environmental Protection Agency (EPA) has previously confirmed the human health safety of both the Cry3Bb1 and CP4 EPSPS proteins (EPA, 2004; EPA, 1996). Further details regarding the safety assessment for the Cry3Bb1 protein and the CP4 EPSPS protein have been published (Hammond et al., 2006a; Hammond et al., 2004; Betz et al., 2000; Harrison et al., 1996).

The second aspect of the safety assessment includes testing for potential unintended effects that may result from insertion of the *cry3Bb1* and *cp4 epsps* coding sequences into the corn genome. "Unintended effects" are defined as effects a crop developer had not anticipated; these could be adverse and could result from pleiotropic effects following insertion of genes into the plant genome or unanticipated changes resulting from the biological activity of introduced protein(s).

With regard to biotechnology, the term "unintended effects," as defined, has been applied in consequence of concerns about the use of this methodology in the development of new plant varieties. For comparison, an equivalent term, "off-types," is used to describe the occurrence of unintended, adverse changes in new plant varieties that are derived by conventional breeding methods.

In order to eliminate "unintended effects," biotechnology takes the same approaches that conventional plant breeders have traditionally used to eliminate "off-types." These approaches include comparative assessments of agronomic, compositional and feeding values, such as comparing MON 88017 with conventionally bred corn varieties (Codex, 2003a; Dybing et al., 2002). The compositional comparison included numerous nutritional components for MON 88017 and conventional corn grown in a variety of geographical locations under different environmental conditions. The field trials and compositional analyses were carried out according to EPA Good laboratory Practice (GLP) standards. Grain was collected from replicated field trials and analyzed for proximates (e.g. protein, fat, and ash), fiber, amino acids, fatty acids, mineral content, vitamins, anti-nutrients and secondary metabolites as recommended by OECD (2002). Forage was also collected and analyzed for proximates and fiber. All comparisons showed that MON 88017 had similar agronomic characteristics (phenotype, yield, etc.) and composition as conventional corn hybrids grown in the same field trial locations (Sidhu and Brown, 2004; McCann et al., 2007).

Although there is a comprehensive set of studies that confirm the absence of unintended effects and the compositional equivalence of MON 88017 corn to existing conventional hybrids, animal feeding studies can also been undertaken to provide further assurance of nutritional wholesomeness and safety (EFSA, 2004). These studies include nutritional feeding trials in poultry to demonstrate that MON 88017 corn grain supports the growth of farm animals comparable to that of near isogenic conventional counterparts (Taylor et al., 2005).

The 13-week "safety assurance" study described here was carried out in rats using toxicological response variables to assess any meaningful differences between MON 88017 and control grain. This study provides added assurance of the safety of MON 88017 grain for human consumption.

2. Materials and methods

The study design was adapted from OECD Guideline No. 408 (1998) and was conducted in general compliance with OECD Good Laboratory Practice (GLP) guidelines (OECD, 1997b) at WIL Research Laboratories, LLC (Ashland, OH, US). With regard to the adaptation of OECD Guideline No. 408, those parts of the guideline were used that were appropriate for the testing of whole food. Most of the toxicology response variables that are normally assessed were included, in addition to which the animal group sizes were twice (20/sex per dose) guideline recommendations (10/sex/dose). Neurobehavioral assessments (such as motor activity, hind limb grip strength, etc.) were not performed as they were not considered appropriate for test articles like corn grain that has no history of producing neurotoxicological effects. Nevertheless, detailed clinical observations, including checking for adverse behavioral signs, open field observations, and the like were performed.

2.1. Animals and maintenance

Male and female Sprague-Dawley-derived rats (Crl:CD®(SD)IGS BR) from Charles River Laboratories (Raleigh, NC, US) were approximately six weeks old at the start of the study. Rats were housed individually and provided food and water *ad libitum*. The testing facility provided appropriate environmental conditions (22 ± 3 °C room temperature, 12-h light/dark cycle, 33.7–57.1% humidity, and approximately 10 air changes/hr), and cage rack location within the animal room was rotated weekly.

2.2. Test and control substances

Corn grain from MON 88017 and its near isogenic control (having the same background genetics but lacking event MON 88017) were grown at field test sites in 2004 in Madison County, IL in compliance with United States Department of Agriculture/Animal Plant Health Inspection Service (USDA/APHIS) guidelines for planting of regulated substances. Only MON 88017 corn was sprayed with glyphosate formulation at commercial application rates; the near isogenic and reference controls were not treated with glyphosate since they would be susceptible to the chemical's herbicidal activity. The grain samples used for diet preparation were analyzed for nutrient components, pesticide residues (Covance Laboratories, Madison, WI, US), including glyphosate (Monsanto Company, St. Louis, MO, US), and mycotoxins (Romer Labs, Union, MO, US). The identity of MON 88017 corn grain was confirmed by lateral flow analysis; the near isogenic control and reference lines served as the negative control in the assay.

Six conventional corn hybrids representing a diversity of corn germplasm were also planted, in Stark County, IL; Hancock County, IN; Fayette County, OH; and Honolulu County, HI, to serve as reference controls for the rat feeding study. Variables measured in rats fed these reference hybrids 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.

2.3. Experimental diets

Diets containing test (MON 88017) grain, its near isogenic control grain (referred to hereafter simply as "control grain"), and the reference control grain (referred to hereafter simply as "reference grain") were formulated by Purina TestDiet (Richmond, IN, US) to be nutritionally and compositionally comparable to PMI Certified Rodent LabDiet[3] 5002. Many toxicology laboratories use this particular diet (PMI Certified Rodent LabDiet 5002) in rodent feeding studies. MON 88017, the control and reference grain were ground and incorporated into the diets. For the test diets, grain incorporation was 11% w/w of MON 88017 plus 22% w/w of the control grain or 33% w/w of MON 88017. For the control diet, control grain incorporation was 33% w/w. Grain from the six reference hybrids was added to diets at a level of 33% w/w. The order of diet preparation was the reference diets were formulated first, then the control diet, and the test diets last. Following diet preparation, samples of all nine diets were analyzed (Covance Laboratories, Madison, WI, US) to confirm that formulated diets met PMI specifications for 5002 rodent diet.

All diets were also tested for the presence of MON 88017 grain using lateral flow strips to confirm the presence of the Cry3Bb1 protein in diets containing the test grain and its absence in diets containing control and reference grain. CP4 EPSPS protein was not used as a marker for the presence of MON 88017 grain in the diets.

2.4. Experimental design and treatment

Following acclimation to laboratory conditions, animals were assigned to one of the nine experimental groups (20/sex/group) by stratified randomization so that mean body weights did not differ significantly ($p < 0.05$) among treatment groups.

[2] Roundup is a registered trademark of Monsanto Company, St. Louis, Missouri, USA.

[3] PMI Certified Lab Diet 5002 is a registered trademark of Purina Mills, Inc.

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 weights were obtained one day prior to group allocation and weekly thereafter. Individual food consumption was determined weekly. Animals continued on test, control, and reference diets for a minimum of 90 days.

2.6. Clinical pathology

Blood was collected under light isoflurane anesthesia via the vena cava from 10 rats/sex/group just prior to terminal sacrifice. Animals were fasted overnight (16–18 h) but did have access to water.

2.6.1. Hematology

Endpoints measured included red blood cell count (RBC), total leukocyte (WBC) and differential (NEU, LYM, etc.) counts, platelet count (PLT), reticulocyte counts, 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 (EDTA) and hematology parameters were measured using Bayer Advia® 120 Hematology System (Tarrytown, NY, US). Prothrombin time (PT) and activated partial thromboplastin time (APTT) were determined from plasma from whole blood collected using sodium citrate as an anticoagulant and measured using an MLA Electra 1400 °C™ (Biomedical Lab Center, Longwood, FL, US) automated coagulation system.

2.6.2. Serum chemistry

Endpoints measured or calculated included albumin (ALB), globulin (GLB), total protein (TP), blood urea nitrogen (BUN), total bilirubin (TBIL), glucose (GLU), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), creatinine (CREA), albumin/globulin ratio (A/G ratio), total cholesterol (CHOL), calcium (Ca), phosphorus (P), chloride (Cl), sodium (Na), potassium (K), and triglycerides. Serum chemistry parameters were measured using a Hitachi 912 clinical analyzer (Basel, Switzerland).

2.6.3. Urinalysis

Using metabolism cages, urine was collected overnight from the same rats used for blood collection. Protein, pH, occult blood, ketones, leukocytes, bilirubin, glucose, nitrites, and urobilinogen were assayed in urine samples with MULTISTIX® reagent strips and a CLINITEK® 200+ urinalysis strip reader (Ames Company, Elkhart, IN, US). Urine volume was measured. Urine specific gravity was determined using an ATAGO Urine Specific Gravity Refractometer (NSG Pression Cells, Inc., Farmington, NY, US). Urine color and appearance were determined by inspection. Sediment, derived from centrifuging the urine sample, was examined microscopically.

2.7. Pathology

At the end of the 13-week exposure period to test, control and reference diets, all animals were anesthetized with CO_2, sacrificed by exsanguination, and given a complete gross pathological examination. Organs weighed were: Adrenals, brain, heart, kidneys, liver, spleen, testes (with epididymides)/ovaries (with oviducts), thymus, and thyroid with parathyroids, with paired organs being weighed together. Tissues collected were: Aorta, adrenals, bone marrow smear, brain, epididymides, esophagus, eyes (with optic nerve), heart, intestine (ileum, jejunum, duodenum, colon, cecum), kidneys, larynx, lesions or abnormal masses, liver, lungs (with mainstem bronchi), lymph nodes (mandibular and mesenteric), nasal cavity, ovaries (with oviducts), pancreas, peripheral nerve (sciatic), pharynx, pituitary, prostate, rectum, salivary glands (mandibular), seminal vesicles, skeletal muscle (rectus femoris), skin (with mammary tissue), spinal cord (three levels), spleen, sternum with marrow, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus (corpus and cervix), and vagina. Following collection, tissues were placed directly into 10% neutral buffered formalin for fixation.

Selected tissues (adrenal glands, brain, epididymides, stomach, duodenum, jejunum, ileum, colon, rectum, heart, kidneys, liver, lymph nodes (mesenteric), ovaries with oviducts, pancreas, spleen, testes, thymus, and thyroid (with parathyroids, if present), gross lesions) representing the major organs/systems from all animals fed 33% w/w MON 88017 and control grain were processed, embedded in paraffin, sectioned (approximately 4 μm), stained with hematoxylin and eosin using standard histological methods, and examined by a board-certified veterinary pathologist using light microscopy.

2.8. Statistical analysis

Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test-article treated group to the control group by sex. These are presented in the text and tables as statistically significantly different at "$p < 0.01$" or "$p < 0.05$." Each group mean is presented with the standard deviation (SD) and the number of animals (N) used to calculate the mean. Body weight, body weight change, food consumption, clinical pathology, and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed statistically significant ($p < 0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test-article treated groups to the control group. Clinical pathology values for white blood cell types that occur at a low incidence (*i.e.*, monocytes, eosinophils and basophils) and histopathology data were not subjected to statistical analyses. The purpose of the reference control population data is that it is intended to be used like laboratory historical control data is typically used, to help interpret the biological relevance of statistical differences that may have been observed between the test and near isogenic control groups.

3. Results

3.1. Analytical

Compositional, contaminant, and nutritional analysis of the experimental diets showed that they met the specifications for Certified Rodent LabDiet 5002 established by PMI (data not shown but available upon request). The levels of heavy metals, aflatoxins, chlorinated, organophosphate insecticides, and glyphosate were all below detection limits. The identity of the test diet was confirmed by the detection of the Cry3Bb1 protein in the diet by lateral flow analysis. The same analytical procedure was used to confirm the absence of Cry3Bb1 protein in control and reference diets.

3.2. Survival and clinical observations

All of the 360 animals were healthy and appeared normal during the course of the study. There were no changes noted over the duration of the study in behavior, activity, posture, gait, or external appearance in any of the groups in either sex (data not shown).

3.3. Body weight and food consumption

Overall, body weight (Fig. 1) and body weight gain (data not shown) were comparable and not statistically different for the male and female MON 88017, control, and reference groups. Final body weights for males were 510 ± 51.0, 511 ± 46.0, and 523 ± 52.3 g for 11% MON 88017, 33% MON 88017, and control males, respectively. For females, the final body weights were 277 ± 33.4, 283 ± 23.9, and 277 ± 18.9 g for 11% MON 88017, 33% MON 88017, and control, respectively. Pooled reference control final body weights were 507 ± 45.2 g for males and 285 ± 22.0 g for females. Food consumption was generally similar between test, control, and reference groups with no consistent statistically significant differences observed (Fig. 2).

3.4. Hematology

There were no adverse, test-article related effects on hematology parameters (Tables 1 and 2). A statistically significant ($p < 0.05$) increase in the absolute count ($10^3/\mu l$)for neutrophils was noted in the high-dose MON 88017 group females (0.85 ± 0.24) compared with the control group (0.58 ± 0.25). Neutrophil counts, calculated as the percentage of the total leukocyte counts, were not, however, statistically significantly different ($p < 0.05$) between the MON 88017 high-dose (13.2 ± 3.33%) and control (9.5 ± 3.88%) female groups. The absolute and percentage counts for neutrophils in the high dose females approximated the historical control means for these values (0.90 ± 0.49 and 13.4 ± 6.05, respectively) for the laboratory for the time period 1999 through July 2005, which time period encompasses the performance of this study (March–June 2004). Furthermore, there was no difference noted in the neutrophil counts in the MON 88017 high-dose group males compared with controls. Additionally, there were no other differences noted in any other hematological parameters between treatment and control groups, and there were no clinical or histo-

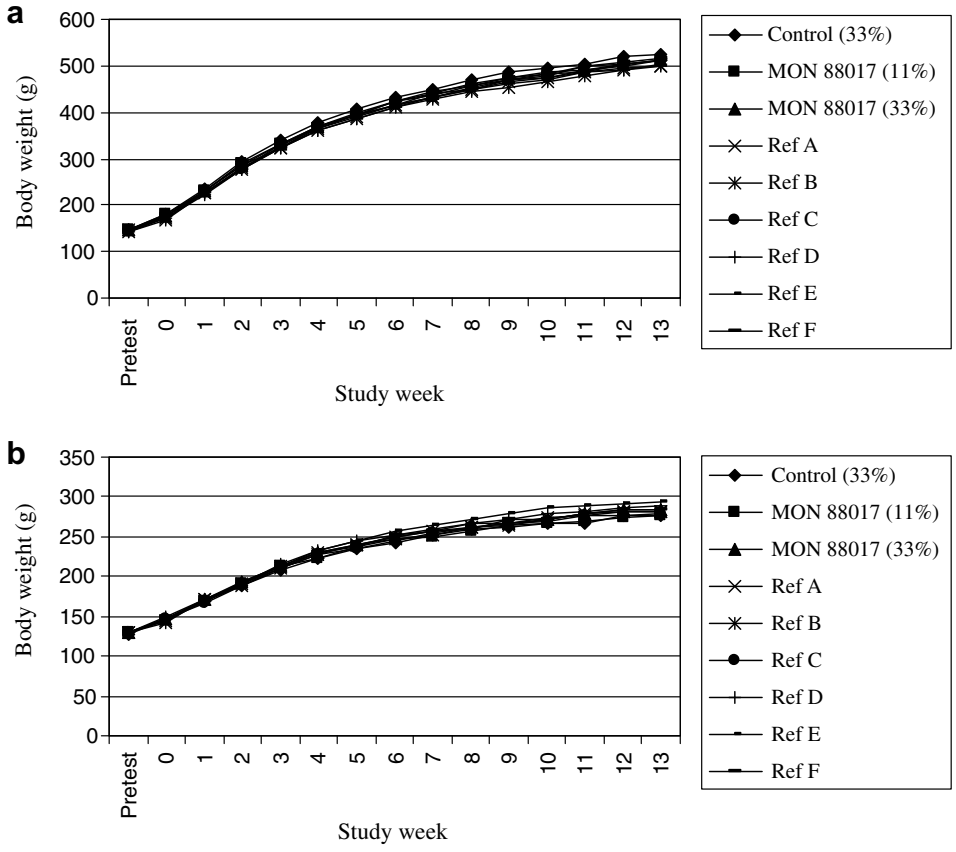

Fig. 1. Mean (a) male and (b) female body weights.

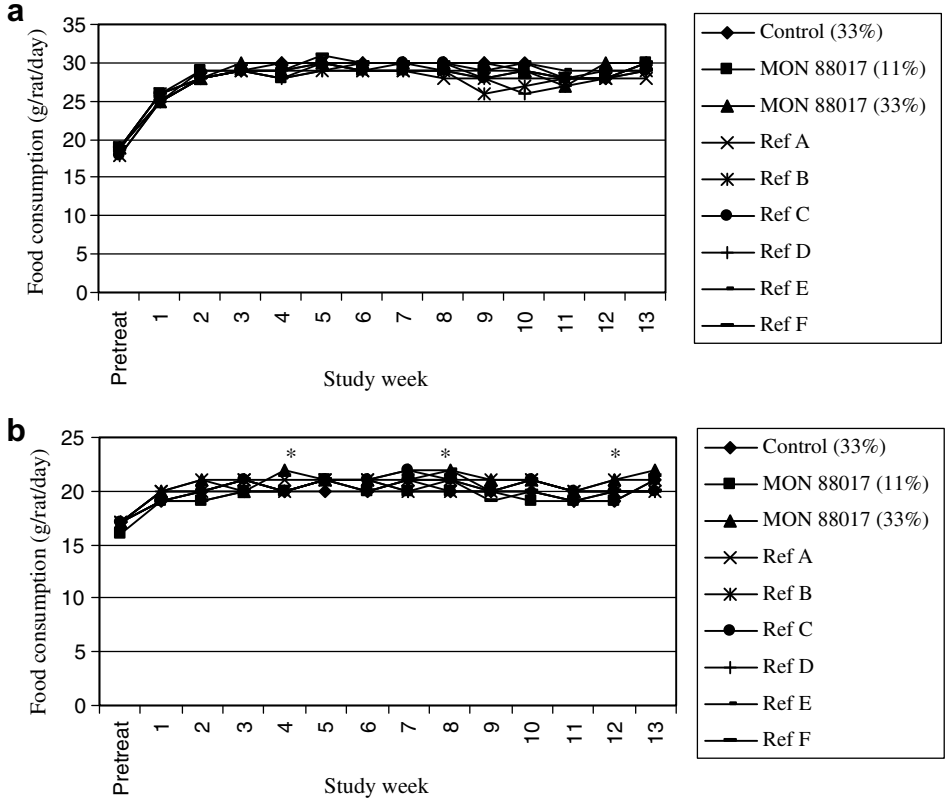

Fig. 2. Mean (a) male and (b) female food consumption. * Statistically significantly different from control, $p < 0.05$.

Table 1
Hematology mean values ± SD[a] in male rats following 13 weeks of exposure to MON 88017

Parameter	N[b]	Near isogenic control	11% w/w MON 88017	33% w/w MON 88017	Reference control[c]
WBC (10^3/μl)	10	10.09 ± 3.11	10.09 ± 3.31	8.78 ± 2.35	9.82 ± 2.88
NEU (10^3/μl)	10	1.17 ± 0.39	1.26 ± 0.72	1.03 ± 0.39	1.11 ± 0.41
NEU (%)	10	12.2 ± 4.55	12.3 ± 4.97	11.8 ± 3.57	11.5 ± 3.52
LYM (10^3/μl)	10	8.34 ± 2.92	8.32 ± 2.86	7.20 ± 1.94	8.13 ± 2.52
RBC (10^6/μl)	10	8.51 ± 0.54	8.24 ± 0.35	8.36 ± 0.55	8.48 ± 0.40
HGB (g/dl)	10	14.9 ± 0.68	14.780.60	14.680.61	14.9 ± 0.64
HCT (%)	10	44.9 ± 3.06	43.5 ± 2.63	43.4 ± 3.39	44.4 ± 2.75
MCV (fl)	10	52.7 ± 1.80	52.8 ± 2.51	51.8 ± 2.09	52.4 ± 2.12
MCH (pg)	10	17.5 ± 0.69	17.8 ± 0.60	17.5 ± 0.68	17.6 ± 0.58
MCHC (g/dl)	10	33.3 ± 0.84	33.7 ± 0.91	33.8 ± 1.26	33.5 ± 0.91
PLT (10^3/μl)	10	1029 ± 157	980 ± 99	1074 ± 163	1014 ± 111
PT (sec)	10	14.2 ± 0.41	14.1 ± 0.51	14.2 ± 0.87	14.0 ± 0.59
APTT (sec)	10	23.3 ± 3.81	23.8 ± 2.29	23.0 ± 2.88	21.7 ± 2.45

There were no statistically significant differences.
[a] Standard deviation.
[b] $N = 10$ for the near isogenic control and MON 88017 treatment groups.
[c] Pooled data from six reference control groups, $N = 60$.

Table 2
Hematology mean values ± SD[a] in female rats following 13 weeks of exposure to MON 88017

Parameter	N[b]	Near isogenic control	11% w/w MON 88017	33% w/w MON 88017	Reference control[c]
WBC (10^3/μl)	10	6.37 ± 2.04	5.89 ± 1.93	6.45 ± 1.20	6.42 ± 1.74
NEU (10^3/μl)	10	0.58 ± 0.25	0.55 ± 0.24	0.85 ± 0.24*	0.65 ± 0.35
NEU (%)	10	9.5 ± 3.88	10.0 ± 5.43	13.2 ± 3.33	10.4 ± 4.53
LYM (10^3/μl)	10	5.48 ± 1.85	5.03 ± 1.84	5.25 ± 1.02	5.42 ± 1.55
RBC (10^6/μl)	10	7.72 ± 0.35	7.67 ± 0.22	7.76 ± 0.47	7.74 ± 0.39
HGB (g/dl)	10	14.4 ± 0.55	14.1 ± 0.29	14.3 ± 0.74	14.3 ± 0.65
HCT (%)	10	41.6 ± 2.24	41.1 ± 1.57	41.2 ± 2.63	41.8 ± 2.65
MCV (fl)	10	53.9 ± 1.45	53.6 ± 2.39	53.1 ± 1.45	54.0 ± 1.64
MCH (pg)	10	18.7 ± 0.47	17.4 ± 0.59	18.5 ± 0.49	18.5 ± 0.51
MCHC (g/dl)	10	34.6 ± 0.72	34.4 ± 0.89	34.8 ± 0.54	34.3 ± 0.85
PLT (10^3/μl)	10	1038 ± 121	1002 ± 68	1118 ± 214	1053 ± 125
PT (sec)	10	13.7 ± 0.58	13.7 ± 0.44	13.5 ± 0.45	13.6 ± 0.49
APTT (sec)	10	19.0 ± 2.74	19.4 ± 2.66	18.7 ± 2.01	17.5 ± 2.08

Statistically significant difference * $P < 0.05$.
[a] Standard deviation.
[b] $N = 10$ for the near isogenic control and both MON 88017 treatment groups.
[c] Pooled data from six treatment groups, $N = 60$ (except PT and APTT, where $N = 59$).

pathological observations to account for the increase in absolute neutrophil counts in high-dose females. Therefore, the difference in the absolute neutrophil counts observed in high-dose female rats was considered an incidental statistical finding that was unrelated to treatment.

3.5. Serum chemistry, urinalysis,

Serum chemistry (Tables 3 and 4) and urinalysis values (data not shown) were not adversely affected by test-article administration. There were no significant differences ($p < 0.05$) when control and test-article treated groups were compared.

3.6. Organ weights

Organ weights were not adversely affected by test-article administration. There were no significant differences ($p < 0.05$) in absolute or organ to body and organ to brain weight ratios when the control and test-article treated groups were compared. Data for organ to body weight ratios is presented in Table 5.

3.7. Pathology

At necropsy, no gross (data not shown) or microscopic lesions (Table 6) were observed that were considered to be test article related. The few findings that were observed were randomly distributed among all groups, including controls, and were of the type commonly observed in rats of this age and strain and were, therefore, considered spontaneous and/or incidental in nature.

4. Discussion

New biotechnology-derived corn varieties can be produced by several different methods. In the past, the coding sequence for single traits such as insect protection (YieldGard Cornborer) or herbicide tolerance (Roundup Ready) were introduced into the corn genome through microparticle bombardment of corn cells. Results of 90 day rat feeding studies using corn varieties transformed by this method have been published (Hammond et al., 2004; Hammond et al., 2006b). These varieties can also be crossed by conventional breeding to generate "stacked" corn products which contain both traits, such as insect protection and herbicide tolerance. Another way to introduce two traits into the same corn variety is to include coding sequences for both traits on the same plasmid or vector (called vector stack), and introduce them into the corn genome. Microparticle bombardment was used to introduce coding sequences for Cry3Bb1 protein (corn rootworm protection) and NPTII (selectable marker) into the YieldGard Rootworm variety which was fed to rats in a 90 day rat feeding study (Hammond et al., 2006a). The MON 88017 variety is also a "vector stack", like the YieldGard Rootworm variety, but used *Agrobacterium*- mediated transformation of corn cells to introduce the coding sequences for Cry3Bb1 and CP4 EPSPS proteins.

Table 3
Serum chemistry mean values ± SD[a] in male rats following 13 weeks of exposure to MON 88017

Parameter	N[b]	Control	11% w/w MON 88017	33% w/w MON 88017	Reference control[c]
ALP (U/L)	10	92 ± 20.3	81 ± 20.4	93 ± 15.5	96 ± 20.8
ALT (U/L)	10	43 ± 10.1	39 ± 9.0	45 ± 8.6	49 ± 14.9
AST (U/L)	10	83 ± 15.8	78 ± 17.9	81 ± 13.3	89 ± 19.4
GGT (U/U)	10	0.7 ± 0.51	0.9 ± 0.92	0.5 ± 0.28	0.7 ± 0.39
BUN (mg/dl)	10	15.6 ± 1.82	15.0 ± 1.65	15.5 ± 1.35	15.2 ± 2.06
CREA (mg/dl)	10	0.3 ± 0.08	0.3 ± 0.10	03 ± 0.07	0.3 ± 0.08
TBIL (mg/dl)	10	0.1 ± 0.05	0.1 ± 0.04	0.1 ± 0.03	0.1 ± 0.05
TP (g/dl)	10	7.0 ± 0.54	6.7 ± 0.42	6.7 ± 0.42	6.9 ± 0.43
ALB (g/dl)	10	4.3 ± 0.25	4.2 ± 0.23	4.2 ± 0.20	4.2 ± 0.24
A/G	10	1.59 ± 0.18	1.67 ± 0.15	1.67 ± 0.15	1.60 ± 0.18
GLOB (g/dl)	10	2.7 ± 0.36	2.5 ± 0.27	2.5 ± 0.26	2.7 ± 0.29
GLU (mg/dl)	10	158 ± 43.1	144 ± 42.1	143 ± 27.1	142 ± 31.7
Ca (mg/dl)	10	11.3 ± 0.75	11.1 ± 0.73	10.9 ± 0.70	11.1 ± 0.58
P (mg/dl)	10	8.9 ± 1.27	8.6 ± 1.31	8.3 ± 1.67	8.8 ± 1.10
Na (mmol/L)	10	147 ± 2.5	147 ± 2.4	146 ± 3.1	147 ± 2.0
Cl (mmol/L)	10	104 ± 2.0	104 ± 1.2	104 ± 1.7	104 ± 1.4
K (mmol/L)	10	5.74 ± 1.05	5.19 ± 1.17	5.46 ± 0.99	5.44 ± 1.17
CHOL (mg/dl)	10	61 ± 12.0	54 ± 10.5	56 ± 16.5	58 ± 12.7
TRIGL (mg/dl)	10	66 ± 29.5	62 ± 22.9	65 ± 33.0	59 ± 27.9

There were no statistically significant differences.
[a] Standard deviation.
[b] $N = 10$ for the near isogenic control and both MON 88017 treatment groups (except GGT, where $N = 4$, 2, and 4 for control, 11% test substance, and 33% test substance groups, respectively).
[c] Pooled data from six treatment groups, $N = 60$ (except GGT where $N = 17$ because the GGT levels for the sera of many of the animals was below the limit of detection of the instrument used for the analyses).

Table 4
Serum chemistry mean values ± SD[a] in female rats following 13 weeks of exposure to MON 88017

Parameter	N[b]	Control	11% w/w MON 88017	33% w/w MON 88017	Reference control[c]
ALP (U/L)	10	57 ± 18.4	46 ± 8.0	57 ± 11.3	56 ± 13.3
ALT (U/L)	10	35 ± 5.4	44 ± 31.7	52 ± 38.1	43 ± 43.5
AST (U/L)	10	79 ± 16.7	87 ± 36.8	102 ± 57.4	88 ± 52.6
GGT (U/U)	10	1.0 ± 0.56	0.7 ± 0.47	0.8 ± 0.47	1.0 ± 0.74
BUN (mg/dl)	10	15.8 ± 2.30	15.4 ± 1.68	16.0 ± 2.46	15.0 ± 2.11
CREA (mg/dl)	10	0.3 ± 0.06	0.3 ± 0.10	0.3 ± 0.06	0.3 ± 0.08
TBIL (mg/dl)	10	0.2 ± 0.05	0.2 ± 0.05	0.2 ± 0.04	0.2 ± 0.05
TP (g/dl)	10	7.2 ± 0.39	7.2 ± 0.59	7.1 ± 0.27	7.2 ± 0.57
ALB (g/dl)	10	4.7 ± 0.41	4.8 ± 0.50	4.6 ± 0.40	4.7 ± 0.37
A/G	10	1.97 ± 0.28	1.94 ± 0.25	1.91 ± 0.32	1.86 ± 0.23
GLOB (g/dl)	10	2.4 ± 0.23	2.5 ± 0.21	2.5 ± 0.32	2.6 ± 0.34
GLU (mg/dl)	10	127 ± 8.9	141 ± 35.2	127 ± 22.5	132 ± 20.6
Ca (mg/dl)	10	11.2 ± 0.48	11.2 ± 0.91	11.1 ± 0.36	11.2 ± 0.72
P (mg/dl)	10	8.2 ± 0.97	8.7 ± 1.41	8.1 ± 1.40	8.9 ± 1.88
Na (mmol/L)	10	147 ± 2.4	146 ± 2.0	146 ± 1.9	147 ± 3.0
Cl (mmol/L)	10	105 ± 1.7	106 ± 1.5	104 ± 1.3	10481.4
K (mmol/L)	10	5.18 ± 0.83	5.43 ± 1.55	5.04 ± 0.81	5.32 ± 1.17
CHOL (mg/dl)	10	63 ± 11.0	65 ± 16.8	64 ± 13.9	67 ± 17.7
TRIGL (mg/dl)	10	40 ± 8.2	40 ± 17.1	32 ± 7.8	36 ± 13.3

There were no statistically significant differences.
[a] Standard deviation.
[b] $N = 10$ for the near isogenic control and both MON 88017 treatment groups (except GGT, where $N = 5$, 5, and 4 for control, 11% test substance, and 33% test substance groups, respectively).
[c] Pooled data from six treatment groups, $N = 60$ (except GGT where $N = 28$ because the GGT levels for the sera of many of the animals was below the limit of detection of the instrument used for the analyses).

During the course of this study, animals fed MON 88017 corn grain in the diet had similar body weights, body weight gains, and food consumption when compared to animals fed diets containing control grain. In addition, there were no test-article related differences in other measured toxicological response variables such as hematology, serum chemistry, and urinalysis parameters for MON 88017 corn fed animals. There were no test-article related changes in organ weights or gross and microscopic pathology.

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

Table 5
Organ/body weight mean values ± SD[a] (%) in male and female following 13 weeks of exposure to MON 88017

Parameter	N[b]	Control	11% w/w MON 88017	33% w/w MON 88017	Reference control[c]
Males					
Adrenals	20	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Brain	20	0.43 ± 0.04	0.44 ± 0.04	0.43 ± 0.04	0.4480.04
Heart	20	0.34 ± 0.03	0.34 ± 0.03	0.34 ± 0.03	0.34 ± 0.04
Thyroid	20	0.0180.00	0.0180.00	0.01 ± 0.00	0.01 ± 0.00
Kidney	20	0.65 ± 0.07	0.66 ± 0.04	0.66 ± 0.06	0.65 ± 0.06
Liver	20	2.75 ± 0.21	2.78 ± 0.22	2.72 ± 0.19	2.72 ± 0.21
Spleen	20	0.17 ± 0.03	0.17 ± 0.02	0.17 ± 0.02	0.17 ± 0.02
Thymus	20	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.07 ± 0.02
Testes	20	0.71 ± 0.09	0.72 ± 0.08	0.74 ± 0.09	0.74 ± 0.08
Females					
Adrenals	20	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Brain	20	0.73 ± 0.05	0.75 ± 0.09	0.73 ± 0.07	0.72 ± 0.05
Heart	20	0.40 ± 0.05	0.40 ± 0.04	0.40 ± 0.04	0.39 ± 0.04
Thyroid	20	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Kidney	20	0.70 ± 0.04	0.72 ± 0.07	0.72 ± 0.04	0.70 ± 0.05
Liver	20	2.79 ± 0.21	2.83 ± 0.17	2.82 ± 0.18	2.80 ± 0.18
Spleen	20	0.20 ± 0.02	0.20 ± 0.03	0.21 ± 0.03	0.20 ± 0.02
Thymus	20	0.10 ± 0.02	0.10 ± 0.02	0.09 ± 0.02	0.10 ± 0.02
Ovaries	20	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01

There were no statistically significant differences.
[a] Standard deviation.
[b] $N = 10$ for the near isogenic control and both MON 88017 treatment groups.
[c] Pooled data from six treatment groups, $N = 118–120$.

Table 6
Microscopic findings in male and female following 13 weeks of exposure to MON 88017

Tissue	Microscopic finding	Control males $N = 20$	MON 88017 33% males $N = 20$	Control females $N = 20$	MON 88017 33% females $N = 20$
Adrenal	Vacuolation, cytoplasmic	1	2	0	0
Epididymides	Infiltrate, lymphocyte	6	6	N/A	N/A
Heart	Cardiomyopathy	10	10	5	6
Kidney	Infiltrate, lymphocyte	9	6	8	7
	Hydronephrosis	1	0	0	0
	Cyst, cortical and/or medullary	2	0	1	0
	Nephropathy, chronic progressive	6	8	2	5
	Mineralization, pelvic	0	0	1	0
Liver	Infiltrate, mononuclear	20	20	20	20
	Necrosis	0	1	1	0
Ovaries	Cyst	N/A	N/A	2	0
Pancreas	Infiltrate, lymphocyte	4	2	4	5

Findings in other tissues with an incidence of 1/20 are not reported. No gross lesions were observed.

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 88017 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 comparable responses of rats fed MON 88017 grain to rats fed control grain supports the absence of untoward unintended effects in MON 88017 corn as confirmed in comprehensive agronomic (Sidhu and Brown, 2004) and compositional (McCann et al., 2007) studies. Furthermore, the nutritional value of MON 88017 grain was compared to control grain in a 42-day broiler chicken performance study with birds fed up to 60% grain in the diet (Taylor et al., 2005). No differences in growth performance were observed indication MON 88017 grain had comparable nutritional value to control grain.

The absence of adverse unintended effects observed in this 90 day rat feeding study are consistent with the results of other published studies with various biotech crops (Hammond et al., 2004, 2006a,b; Malley et al., 2007; Dryzga et al., 2007; MacKenzie et al., 2007; Schroder et al., 2007). Previous publications have also demonstrated that there are substantial safety margins when potential human dietary intake of corn grain containing these proteins is compared to dietary exposure of rodents fed the grain in 90-day rat feeding studies (Hammond et al., 2004, 2006a,b).

5. Conclusion

No adverse health effects were noted when diets containing grain from MON 88017 were administered to rats. Consistent with agronomic, compositional and farm animal feeding studies, the 90-day rat study did not detect unintended effects in the grain. These results demonstrate that MON 88017 is as safe and nutritious as conventional corn hybrids.

Conflict of Interest Statement

There are no conflicts of interest for any of the authors of this article.

References

Betz, F.S., Hammond, B.G., Fuchs, R.L., 2000. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. Regulatory Toxicology and Pharmacology 32, 156–173.

Borzelleca, J.F., 1996. A proposed model for safety assessment of macronutrient substitutes. Regulatory Toxicology and Pharmacology 23, S15–S18.

CAST, 2001. Evaluation of the US Regulatory Process for Crops Developed through Biotechnology. Abramson, S.H., Dyer, W.E., Faust, M.A., Harlander, S.K., Hefle, S.L., Munro, I.C., Rice, M.E. (Task Force). Issue Paper, No. 19, Council for Agricultural Science and Technology, Ames, IA.

Chesson, A., 2001. Assessing the safety of GM food crops. Hester, R.E., Harrison, R.M. (Eds.), Reprinted from Food Safety and Food Quality, Issues in Environmental Science and Technology No. 15, pp. 1–24.

Cockburn, A., 2002. Assuring the safety of genetically modified (GM) foods: the importance of an holistic, integrative approach. Journal of Biotechnology 98, 79–106.

Codex, 2003a. Joint FAO/WHO Food Standards Programme. Codex Alimentarius Commission. Report of the Fourth Session of the Codex *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology. Alinorm 03/34A. <http://www.codexalimentarius.net/web/archives.jsp?year=03>.

Codex, 2003b. Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAG/GL 45-2003).

Dunnett, C.W., 1964. New tables for multiple comparisons with a control. Biometrics 20, 482–491.

Dryzga, M., Yano, B., Andrus, A., Mattson, J., 2007. Evaluation of the safety and nutritional equivalence of a genetically modified cottonseed meal in a 90-day dietary toxicity study in rats. Food and Chemical Toxicology 45, 1994–2004.

Dybing, E., Doe, J., Groten, J., Kleiner, J., O'Brien, J., Renwick, A.G., Schlatter, J., Steinberg, P., Tritscher, A., Walker, R., Younes, M., 2002. Hazard characterization of chemicals in food and diet: dose response, mechanisms and extrapolation issues. Food and Chemical Toxicology 40, 237–282.

EFSA, 2004. Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed, 8 November 2004. Available from: <http://www.efsa.eu.int/cf/consultation.cfm>.

Elias, P., 1980. The wholesomeness of irradiated food. Ecotoxicology and Environmental Safety 4, 172–183.

EPA, 1996. Plant pesticide inert ingredient CP4 enolpyruvylshikimate-3-d and the genetic material necessary for its production in all plants; Final rule. US Environmental Protection Agency, Federal Register 61, 40338–40340.

EPA, 2004. *Bacillus thuringiensis* Cry3Bb1. Exemption from the requirement of a tolerance; Final rule. US Environmental Protection Agency, Federal Register 69, 16809–16814.

FAO, 1996. Biotechnology and Food Safety. Report of a Joint FAO/WHO Consultation, FAO Food and Nutrition Paper 61, Rome, Italy, 30 September–4 October, 1996.

FAO/WHO, 2000. Safety aspects of genetically modified foods of plant origin. Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology Consultations and Workshops. WHO/SDE/FOS/00.6 (English). World Health Organization/Food and Agriculture Organization of the United Nations, Geneva, Switzerland, 29 May–2 June 2000.

Hammond, B.G., Rogers, S.G., Fuchs, R.L., 1996. Limitations of Whole Food Feeding Studies in Food Safety Assessment. In: Food Safety Evaluation. OECD Documents, Paris, France, pp. 85–97.

Hammond, B., Dudek, R., Lemen, J., Nemeth, M., 2004. Results of a 13 week safety assurance study with rats fed grain from glyphosate tolerant corn. Food and Chemical Toxicology 42, 1003–1014.

Hammond, B., Lemen, J., Dudek, R., Ward, D., Jiang, C., Burns, M., Nemeth, J., 2006a. Results of a 90-day safety assurance study with rats fed grain from corn rootworm-protected corn. Food and Chemical Toxicology 44, 147–160.

Hammond, B., Dudek, R., Lemen, J., Nemeth, M., 2006b. Results of a 90-day safety assurance study with rats fed grain from corn borer-protected corn. Food and Chemical Toxicology 44, 1092–1099.

Harrison, L.A., Bailey, M.R., Naylor, M.W., Ream, J.E., Hammond, B.G., Nida, D.L., Burnette, B.L., Nickson, T.E., Mitsky, T.A., Taylor, M.L., Fuchs, R.L., Padgette, S.R., 1996. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested *in vitro* and is not toxic to acutely gavaged mice. Journal of Nutrition 126, 728–740.

IFBC, 1990. Biotechnologies and food: assuring the safety of foods produced by genetic modification. International Food Biotechnology Council. Regulatory Toxicology and Pharmacology 12, No. 3.

Kessler, D.A., Taylor, M.R., Maryanski, J.H., Flamm, E.L., Kahl, L.S., 1992. The safety of foods developed by biotechnology. Science 256, 1747–1749.

Kuiper, H.A., Kleter, G.A., Noteborn, H.P.J.M., Kok, E.J., 2001. Assessment of the food safety issues related to genetically modified foods. Plant Journal 27, 503–528.

MacKenzie, S.A., Lamb, I., Schmidt, J., Deege, L., Morrisey, M.J., Harper, M., Layton, R.J., Prochaska, L.M., Sanders, C., Locke, M., Mattsson, J.L., Fuentes, A., Delaney, B., 2007. Thirteen week feeding study with transgenic maize grain containing event DAS-Ø15Ø7-1 in Sprague-Dawley rats. Food and Chemical Toxicology 45, 551–562.

Malley, L., Everds, N., Reynolds, J., Mann, P., Lamb, I., Rood, T., Schmidt, J., Layton, R., Prochaska, L., Hinds, M., Locke, M., Chui, C., Claussen, F., Mattson, J., Delaney, B., 2007. Subchronic feeding study of DAS-59122-7 maize grain in Sprague-Dawley rats. Food and Chemical Toxicology 45, 1277–1292.

McCann, M.C., Trujillo, W.A., Riordan, S.G., Sorbet, R., Bogdanova, N.N., Sidhu, R.S., 2007. Comparison of the forage and grain composition from insect-protected and glyphosate-tolerant MON 88017 corn to conventional corn (*Zea mays* L). Journal of Agriculture and Food Chemistry 55, 4034–4042.

OECD, 1993. Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles, Organisation for Economic Cooperation and Development (OECD), Paris.

OECD, 1997a. Report of the Workshop on the Toxicological and Nutritional Testing of Novel Foods. SG/ICGB(98)1.

OECD, 1997b. Principles of Good Laboratory Practice (C(97) 186/Final), 26 November 1997.

OECD, 1998. Organisation for Economic Cooperation and Development Guidelines (21 September 1998) for the Testing of Chemicals, Health Effects Test Guidelines, Section 408.

OECD, 2002. Consensus document on compositional considerations for new varieties of maize (*Zea mays*): key food and feed nutrients, anti-nutrients and secondary plant metabolites. Organization for Economic Cooperation and Development. OECD Environment Directorate Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, vol. 6.

Pauli, G.H., Takeguchi, C.A., 1986. Irradiation of foods – an FDA perspective. Food Reviews International 2, 79–107.

Schrøder, M., Poulsen, M., Wilcks, A., Kroghsbo, S., Miller, A., Frenzel, T., Danier, J., Rychlik, M., Emani, K., Gatehouse, A., Shu, Q., Engle, K.-H., Altosaar, I., Knudsen, I., 2007. A 90-day safety study of genetically modified rice expressing Cry1Ab protein (*Bacillus thuringiensis* toxin) in Wistar rats. Food and Chemical Toxicology 45, 339–349.

Sidhu, R.S., Brown, S., 2004. Petition for Determination of Nonregulated Status for MON 88017 Corn. Available from: <http://www.aphis.usda.gov/brs/aphisdocs/04-12501p.pdf>.

Snedecor, G.W., Cochran, W.G., 1980. One way classifications: analysis of variance. In: Statistical Methods, Seventh ed. The Iowa State University Press, Ames, IA, pp. 215–237.

Taylor, M.L., Hyun, Y., Hartnell, G.F., Nemeth, M.A., Karunanandaa, K., George, B., Glenn, K.C., 2005. Comparison of broiler performance when fed diets containing MON 88017, MON 88017 x MON 810, control, or commercial corn. Poultry Science 83 (Suppl. 1), 322. (Abstract W39).

WHO, 1991. Strategies for Assessing the Safety of Foods Produced by Biotechnology. Report of a Joint FAO/WHO Consultation. World Health Organization, Geneva.

WHO, 1995. Application of the Principles of Substantial Equivalence to the Safety Evaluation of Foods or Food Components from Plants Derived by Modern Biotechnology. Report of a WHO Workshop, World Health Organization, Geneva. WHO/FNU/FOS/95.1.