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Thirteen week rodent feeding study with grain from molecular stacked trait lepidopteran and coleopteran protected (DP-ØØ4114-3) maize

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ABSTRACT

The results from a subchronic feeding study conducted in Sprague–Dawley rats fed with diets containing grain from 4114 (OECD unique identifier: DP-ØØ4114-3) maize that was untreated (4114) or sprayed in field with glufosinate ammonium (4114GLU) in a design similar to previous studies are reported. The test material, 4114 maize, is a hybrid maize produced by transformation with a DNA construct encoding 4 different transgenic proteins for resistance to lepidopteran pests, coleopteran pests, and tolerance to the herbicidal active ingredient glufosinate ammonium. There were a total of 144 rats divided into 12 groups of 12 rats/sex/group. All experimental diets were formulated by Purina Mills, LLC (St. Louis, MO) in accordance with the standards of Purina Mills Labdiet® Certified Rodent LabDiet® 5002. The incorporation rate of maize grain in all diets was 32% (wt/wt). No biologically significant, treatment related differences in body weight, food consumption, clinical pathology parameters (hematology, blood chemistry, urinalysis, or organ weight) were observed in rats consuming the diets containing 4114 maize grain compared with rats fed conventional maize diets. A number of histologic observations were noted in this study but were background lesions and representative of what would be expected for rats of this age and strain. An independent panel of experts determined certain observations to be spontaneous and not related to the test diet. Accordingly, these results support the conclusion that 4114 maize grain is as safe and nutritious as conventional maize grain.

1. Introduction

Biotechnology has been used to introduce traits such as insect resistance and herbicide tolerance into field crops by transformation with DNA constructs that drive expression of transgenic proteins. Since the mid 1990s, a significant number of biotech crops containing an assortment of insecticidal proteins from the ubiquitous soil bacteria *Bacillus thuringiensis* (Bt proteins) have been introduced into the market. Increasingly, farmers have chosen to plant insect control traits in combination with various herbicide-tolerance traits for improved insect and weed control. Biotech crops have revolutionized agriculture, with high levels of adoption in the USA and around the globe that provide economic gains for farmers, along with substantial environmental benefits. Prior to commercialization, the introduced proteins in genetically modified (GM) plants are subjected to rigorous testing to ensure that they are not allergenic or toxic (Codex, 2003; Delaney et al., 2008). Following transformation and selection, crops containing the transgenic proteins are also subjected to a comprehensive safety assessment based on the concept of substantial equivalence (WHO, 1991, 1995). This has more recently been described as a comparative safety assessment as the focus is on demonstrating that any particular GM crop is "as safe as" its closest conventional non-GM comparator with a history of safe use (FAO, 2008). This includes compositional and agronomic performance comparisons to determine whether the development of a particular GM crop resulted in unintended effects (Herman et al., 2003; McCann et al., 2007; Ridley et al., 2002; Sidhu et al., 2000). Subchronic rodent feeding studies have also been conducted in support of the safety assessment process for a number of individual corn and soybean GM crops (Hammond et al., 2006a,b, 2004; MacKenzie et al., 2007; Malley et al., 2007; Appenzeller et al., 2008; He et al., 2008; Healy et al., 2008). To date, these studies have not identified test diet related adverse effects following dietary exposure to whole grains from commercialized GM crops.

Crop plants with multiple transgenic traits have been developed using breeding methods to cross individual GM lines. Because no new DNA is introduced when crossing a safety assessed GM crop with another safety assessed GM crop additional safety testing

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beyond what was conducted on the original GM events is generally considered unnecessary (ACRE, 2004; De Schrijver et al., 2007; Pilacinski et al., 2011). This is supported by compositional studies (Ridley et al., 2011) as well as feeding studies conducted in rats (Appenzeller et al., 2009) and in livestock species (Taylor et al., 2003a,b,c). It is also possible to introduce multiple traits into a field crop through design of multigene vectors that express more than one transgenic protein, referred to as molecular stacking (see Que et al., 2010).

The current paper reports the results of a subchronic rodent feeding study that was conducted with maize grain obtained from a GM hybrid corn (DP-ØØ4114-3; hereafter referred to as 4114 maize). The transformation plasmid used to produce 4114 maize was designed to express: (i) Cry1F protein for resistance to European corn borers (ECB; *Ostrinia nubilalis* Hübner: Crambidae) and other lepidopteran pests; (ii) Cry34Ab1 and Cry35Ab1 proteins for resistance to corn rootworms and other coleopteran pests (CRW; *Diabrotica virgifera virgifera* LeConte and *Diabrotica barberi* Smith and Lawrence); and (iii) the PAT protein for tolerance to the herbicidal active ingredient glufosinate ammonium. The Cry proteins demonstrate species selective toxicity via pore formation within the gut of sensitive larvae (Piggot and Ellar, 2007; CERA, 2011). The PAT protein detoxifies glufosinate via *N*-acetylation thereby preventing it from inhibiting glutamine synthase which is a critical enzyme required to detoxify ammonia produced during photorespiration and other cellular activities (De Block et al., 1987). None of these proteins are allergenic or toxic (Herman et al., 2003; Hérouet et al., 2005; Ladics et al., 2006; Delaney et al., 2008; Juberg et al., 2009) and no test diet related adverse effects were observed in rodent studies conducted with grain from individual GM hybrid crops in which these proteins were expressed (MacKenzie et al., 2007; Malley et al., 2007; He et al., 2008). Similarly, no evidence of test diet related adverse effects was observed in a subchronic feeding study conducted with grain from a GM maize plant produced through conventional breeding containing all four proteins (Appenzeller et al., 2009). The current study was conducted to support the safety of grain obtained from 4114 maize.

This study was conducted in accordance with OECD guidelines (No. 408; OECD, 1998) for 13 week repeated dose toxicology studies in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). The protocol and study design were reviewed and approved by the DuPont Haskell Institutional Animal Care and Use Committee (IACUC).

In the course of conducting the histopathologic analysis of the tissues slides from this study, amphophilic vacuolar (AV) kidney tumors were observed in two male rats from the group consuming test diet containing grain from 4114 maize. An expert panel of toxicologic pathologists was assembled (Pathology Working Group) to review the tissue slides for purposes of classification and they determined unanimously that the AV tumors were spontaneous and not test diet related. The detailed methods and tissue slides are presented in the accompanying publication (Hardisty et al., submitted for publication).

2. Materials and methods

2.1. Production and analytical characterization of maize grains

4114 maize grain from glufosinate-treated plants (4114GLU) and non-glufosinate-treated plants (4114), non-GM near-isogenic control maize grain (091), and commercially available non-GM hybrid reference maize grains (32D78, 33N29, and 34P88) were grown in isolated field plots in York, Nebraska in the 2010 growing season. The identities of the maize grains used in this study were confirmed using event specific qualitative polymerase chain reaction (PCR) methods for the presence or absence of event DP-ØØ4114-3 and the presence and concentrations of the Cry34Ab1, Cry35Ab1, Cry1F and PAT proteins using antibody specific enzyme-linked immunosorbant assay (ELISA) (Pioneer Hi-Bred International, Inc. Ankeny, IA). Maize grain composition of nutritional proximates, carbohydrates, fiber values, individual amino acids, fatty acids, vitamins, minerals, anti-nutrients and secondary metabolites were determined as previously described (Appenzeller et al., 2009) at EPL-BioAnalytical Services, Inc. (EPL-BAS; Niantic, IL). Additional fatty acid composition (caprylic, capric, lauric, myristic, myristoleic, pentadecanoic, pentadecenoic, palmitoleic, heptadecanoic, heptadecenoic, heptadecadienoic, C18:2 (9,15), nonadecanoic, arachidic, eicosenoic, eicosadienoic, eicosatrienoic, arachadonic, heneicosanoic, behenic, erucic, tricosanoic and lignoceric [percentage of total fatty acids]) and the concentrations of pantothenic acid (mg/100 g), pyridoxine (mg/100 g) and niacin (mg/100 g) were determined by EPL-BAS. Selenium concentration (ppm) was determined by Eurofins Scientific, Inc. (Des Moines, IA). Chlorinated hydrocarbon residues and organophosphates were determined at Columbia Food Laboratories, Inc. (Corbett, OR). Mycotoxin concentrations were determined at Romer Laboratories, Inc. (Union, MO) and the University of Missouri Veterinary and Medical Diagnostic Laboratory (Columbia, MO).

2.2. Diet formulation, composition, and administration

All experimental diets were formulated by Purina Mills, LLC (St. Louis, MO) in accordance with the standards of Purina Mills Labdiet® Certified Rodent LabDiet® 5002 (PMI® 5002; PMI Nutrition International, 2009) and prepared at Purina TestDiet (Richmond, IN). The control diet was prepared with ground 091 maize grain, the test diets with DP-ØØ4114-3 maize grain from glufosinate-treated plants (4114GLU) or untreated plants (4114), and reference diets with maize grain from 32D78, 33N29, or 34P88 plants. The incorporation rate of maize grain in all diets was 32% (wt/wt) which is within the standard range of corn for PMI® 5002 diets. Experimental diets were refrigerated upon arrival at DuPont Haskell Global Centers for Health and Environmental Sciences (Haskell; Newark, DE). Fresh diet was supplied weekly, and animals were fed the experimental diets for at least 91 consecutive days.

2.3. Analytical characterization of experimental diets

Nutritional and contaminant analyses of all experimental diets were conducted as described previously (MacKenzie et al., 2007; Malley et al., 2007; Appenzeller et al., 2008, 2009). Homogeneity of all experimental diets was assessed in samples obtained from the beginning, middle and end of diet production by analysis of Cry34Ab1, Cry35Ab1, Cry1F, and PAT protein concentrations using antibody specific ELISA. Stability of the 4114 and 4114GLU experimental diets was assessed by determination of the concentrations of the aforementioned proteins using antibody specific ELISA in samples collected at the time of first use, following storage under ambient conditions for 1 week and again at the end of the feeding trial (Week 13). Additionally, Cry1F protein activity was assessed in samples of the 4114 and 4114GLU diets obtained at the beginning of the feeding trial using a European corn borer (ECB) bioassay as described previously (MacKenzie et al., 2007). As described previously, the concentrations of the Cry34Ab1 and Cry35Ab1 proteins in maize grain are not high enough to inhibit corn rootworm (CRW) growth, therefore their biological in the test diet was not assessed (Malley et al., 2007).

2.4. Test system

Male and female Crl:CD® (SD)IGS BR rats were obtained from Charles River Laboratories, Inc., (Kingston, NY). Rats were housed individually in solid-bottom caging containing Shepherd's Cob + Plus™ bedding (Shepherd Specialty Papers, Watertown, TN). The animal room was maintained at a temperature of 22 ± 4 °C and a relative humidity of 30–70%, and fluorescent lighting was provided on an approximate 12-h light/dark cycle. Tap water (United Water Delaware, Wilmington, DE) was provided *ad libitum* to all rats. During the pretest period, animals were fed PMI Certified Rodent LabDiet® 5002 *ad libitum*. During the test period, animals were fed the 091, 4114, 4114GLU, 32D78, 33N29 or 34P88 diets *ad libitum*, except prior to blood collection for clinical pathology evaluations and sacrificed when food, but not water, was withheld. Rats approximately 6–8 weeks of age were assigned to groups (n = 12/sex/group) following computerized randomization based on body weight. Body weight and food consumption were determined for all rats twice during the first week of the test period and weekly thereafter. Food efficiency was calculated for the same intervals from the food consumption and body weight data.

2.5. Clinical anatomic pathology

This feeding trial was conducted in accordance with the OECD 408 guideline for a repeated dose 90-day oral toxicity study in rodents (OECD, 1998). All animals were observed for mortality or moribundity twice daily and clinical observations were conducted daily. Ophthalmology and neurobehavioral evaluations, including a functional observational battery (FOB) and motor activity assessment, were conducted for all rats once prior to exposure to the experimental diets and during Week 13 as reported previously (MacKenzie et al., 2007; Malley et al., 2007; Appenzeller et al., 2008). After exposure to the experimental diets for 91–92 days (Males) and

92–93 or 96 days (Females), all surviving rats were fasted at least 15 h prior to blood collection for clinical pathology evaluations and the scheduled sacrifice. Each animal was weighed, anesthetized by inhalation of isoflurane, and sacrificed by exsanguination. All rats were subjected to a complete gross pathology examination during necropsy. Clinical and anatomic pathology assessments were conducted at the end of the feeding interval (Week 14) following overnight fasting. Clinical chemistry variables included serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), sorbitol dehydrogenase (SDH), alkaline phosphatase (ALKP), total bilirubin (BILI), urea nitrogen (BUN), creatinine (CREA), cholesterol (CHOL), triglycerides (TRIG), glucose (GLUC), total protein (TP), albumin (ALB), globulin (GLOB), calcium (CALC), inorganic phosphorus (IPHS), sodium (NA), potassium (K), and chloride (CL). Hematology variables included red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), absolute reticulocyte count (ARET), platelet count (PLT), white blood cell count (WBC), differential white blood cell count (ANEU [neutrophils], ALYM [lymphocytes], AMON [monocytes], AEOS [eosinophils], ABAS [basophils], and ALUC [large unstained cells]; prothrombin time (PT) and activated partial thromboplastin time (APTT). Urinalysis variables included volume (VOL), specific gravity (SG), pH, bilirubin, and urobilinogen (URO), and urine protein concentrations (UMTP), and urine osmolality (UOSM). Histopathologic analysis was conducted on all rats from the 091, 4114 and 4114GLU maize grain groups. All observations and analyses were conducted at DuPont Haskell and details related to individual response variables, methods, tissues evaluated for microscopic pathology evaluation were described in previous publications (MacKenzie et al., 2007; Malley et al., 2007; Appenzeller et al., 2008) with the exception of the serial step sectioning that was conducted on all available kidneys from the control, reference, 4114, and 4114GLU treatment groups as described by Eustis and co-workers (1994).

2.6. Statistical analysis

Comparisons were designed to determine whether differences were attributable to consumption of diets containing the 4114 and 4114GLU maize grain compared to the control diet containing 091 near isogenic maize grain. Data from male and female rats were analyzed separately. Groups of male and female rats consuming diets containing maize grains from non-GM reference plants (32D78, 33N29, and 34P88) were included to assess variability of the response variables evaluated in the current study. Preliminary tests to verify homogeneous variances (Levene's test; Levene, 1960) and normality (Shapiro–Wilks test; Shapiro and Wilk, 1965) were conducted for all continuous data. The use of contrasts retained the within-group and between-group variances of the 091 control and reference groups (32D78, 3N29 and 34P88). With the exception of motor activity and grip strength data, if neither preliminary homogeneity test was significant, a one-way analysis of variance (ANOVA) was conducted using p-adjusted linear contrasts (Snedecor and Cochran, 1967). If one or more of the preliminary tests was significant, Dunn's Type 1 p-adjusted linear contrasts were used (Dunn, 1964). For motor activity and grip strength data, if neither preliminary homogeneity test was significant, a repeated measures analysis of variance (RANOVA) followed by linear contrasts was conducted (Milliken and Johnson, 1984; Hocking, 1985). If one or more of the preliminary tests was significant, a normalizing, variance stabilizing transformation followed by RANOVA (Milliken and Johnson, 1984) or a non-parametric test (Dunn, 1964) was conducted. Descriptive FOB data were analyzed using Fisher's Exact test (Fisher, 1985) with a Bonferroni-Holm correction (Holm, 1979). Where indicated, historical control ranges were obtained from other studies conducted at Haskell within the 10 years preceding this feeding trial using rats of similar species, age and strain.

3. Results

3.1. Characterization of maize grains and diets

All maize grains used in this study contained similar concentrations of nutrients and contaminants and ELISA and event specific PCR demonstrated that autonomy of the different maize lines was properly controlled (data not shown). Similarly, the composition of the rodent diets were comparable regardless of the source of the maize grains used to prepare them (Table 1). Quantitative antibody specific ELISA testing for the Cry1F, Cry34Ab1, Cry 35Ab1, and PAT proteins was conducted on the 4114 and 4114GLU diets to demonstrate that the diets were blended homogenously at the beginning of the study and throughout the course of the in life phase of the study to confirm the stability of these proteins (data not shown). Additionally, a bioassay conducted on European corn borer larvae with the rodent diets at the beginning and end of the in-life phase as described previously (MacKenzie et al., 2007), demonstrated that the Cry1F protein was biologically active (data not shown).

3.2. Clinical and anatomic pathology

3.2.1. Body weights, food consumption and clinical observations

There were no differences in body weight or food consumption between rats consuming diets containing maize grain from the 091 control compared with those containing the 4114 or 4114GLU maize grain (Fig. 1 and 2). No adverse effects were observed in any ophthalmological, clinical observations or neurobehavioral response variables including the FOB and motor activity assessment (data not shown).

3.2.2. Hematology and coagulation

The mean HGB and HCT values in males consuming the 4114 maize grain were greater ($p < 0.05$) than the values from males consuming the 091 control maize grain (Table 2). While the difference was statistically significant, this was not considered to be test diet related or an adverse effect because the difference was small in magnitude and all of the individual HGB (15.6–17.1 g/dL) and HCT (45.8–50.9%) values from the 4114 maize grain group fell within the range of the historical control data of the testing facility.[1] There were no other statistical differences in the male rats and there were no statistical differences in the hematology response variables between females in the 4114 maize grain and the 091 control diet group (Table 3).

There were no statistically significant differences in plasma thromboplastin time (PT) and activated partial thromboplastin time (APTT) in male or female rats consuming the 4114 maize grain diet compared with the 091 control diet (Tables 2 and 3).

3.2.3. Clinical chemistry

No adverse or test diet-related differences in mean clinical chemistry response variables were observed in male or female rats fed the 4114 or 4114GLU test diets compared with the 091 control group (Tables 4 and 5). There were, however, a number of statistical differences that were not considered test diet related or adverse. The mean serum alkaline phosphatase (ALKP) activity was higher ($p < 0.05$) in male rats from the 4114GLU group than the 091 Control group. However, all of the individual ALKP values from the 4114GLU group (82–130 U/L) were within the range of historical control for similarly aged animals[2] and there was no other indication of liver or other organ toxicity in the animals from that group. Therefore, the difference was considered to be spurious, unrelated to consumption of the test diet and not an adverse effect.

The mean creatinine (CREA) concentration was higher ($p < 0.05$) in male rats from the 4114GLU group than in the 091 Control group. However, there were no statistical differences between the 091 control group and any of the other treatment groups and, importantly, no correlative increases in blood urea nitrogen (BUN) in males from the 4114GLU group and no other indicators of kidney toxicity. Therefore, this difference was considered spurious and not related to consumption of the test diet.

Group mean potassium (K) was lower ($p < 0.05$) in female rats from both the 4114 and 4114GLU groups. These statistical differences were also considered to be unrelated to consumption of the test diets and non-adverse as they were minimal in magnitude (94% and 92% of control, respectively) and were not associated with differences in other serum electrolytes (sodium or chloride). Further all of the individual K values in these groups were within the range of individual values in the control or reference diet

[1] Historical control HGB values range from 13.3 to 17.5 g/dl and historical control hct values range from 42.1% to 55.0%.

[2] Historical control ALKP values range from 43 to 158 U/L.

Table 1
Nutritional composition of diets.

Nutritional analysis of rodent diets						
Analyte[a,b]	091	4114	4114GLU	32D78	33N29	34P88
Proximate analytes						
Moisture (%)	11.4	10.9	10.8	11.1	10.8	11.2
Dry matter (%)	88.6	89.1	89.2	88.9	89.2	88.8
Crude protein (% FW)	20.55	20.55	20.64	20.67	20.31	20.3
Carbohydrate (% FW)	57.5	58.0	58.2	57.6	58.3	58.1
Crude fat (% FW)	5.39	5.46	5.36	5.34	5.40	5.40
Ash (% FW)	5.20	5.07	4.98	5.30	5.13	4.98
Crude fiber (% FW)	3.83	4.27	4.39	4.22	4.39	4.28
Neutral detergent fiber (% FW)	11.3	12.8	12.7	12.5	12.4	13.0
Acid detergent fiber (% FW)	5.50	6.15	6.69	5.85	6.09	6.19
Gross energy (kcal/100 g of FW)	401	405	404	402	406	405
Amino acids (%FW)						
Arg	1.12	1.10	1.11	1.15	1.06	1.14
Cys	0.291	0.309	0.305	0.280	0.288	0.283
Gly	0.977	0.928	0.927	0.971	0.896	0.962
His	0.530	0.511	0.517	0.526	0.491	0.527
Ile	0.818	0.808	0.812	0.839	0.818	0.832
Leu	1.63	1.59	1.60	1.65	1.64	1.65
Lys	1.11	1.13	1.12	1.18	1.20	1.14
Met	0.446	0.411	0.435	0.413	0.454	0.398
Phe	0.965	0.929	0.949	0.964	0.898	0.960
Tyr	0.496	0.482	0.490	0.498	0.470	0.500
Thr	0.774	0.763	0.763	0.785	0.757	0.782
Trp	0.218	0.215	0.217	0.212	0.217	0.212
Val	0.964	0.950	0.948	0.985	0.955	0.976
Ser	0.975	0.955	0.962	0.992	0.953	0.984
Asp	1.86	1.98	1.98	2.03	2.04	1.90
Glu	3.74	3.81	3.79	3.96	4.01	3.80
Ala	1.03	1.03	1.03	1.07	1.07	1.05
Pro	1.33	1.25	1.25	1.33	1.30	1.30

[a] Additional nutrients measured: minerals (Ca, Cu, Fe, Mg, Mn, P, K, Na, Zn, Se, Co, Cl, I, Cr, F) and vitamins (folic acid, niacin, pantothenic acid, biotin, choline, A (retinol), beta-carotene, B1 (thiamine), B2 (riboflavin), B6 (pyridoxine), B12, D3, beta-carotene, vitamin E as alpha-, beta, delta-, gamma-, and total tocopherol).

[b] Contaminants measured: heavy metals (As, Cd, Pb, Hg); mycotoxins (aflatoxins B1, B2, G1, and G2, zearalenone, oosporein, ergosine, ergotamine, ergocornine, ergocryptine, ergocristine), deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol, cyclopiazonic acid, fumonisin B1, B2, B3, moniliformin, T-2 toxin; and chemical residues chlorinated hydrocarbons (aldrin, BHC-alpha, -beta, -delta, chlordane, DDT-related compounds, dieldrin, endrin, HCB, heptachlor, heptachlor epoxide, lindane, methoxyclor, mirex, PCB) and organophosphates (diazinon, disulfoton, ethion, malathion, methyl parathion, parathion [ethyl], phorate [thimet], thiodan [endosulfan], carbophenothion [trithion]).

groups (with the exception of 2 rats from the 4114GLU group) and no differences in K values were present in male rats from the 4114 or 4114GLU groups.

3.2.4. Urinalysis

There were no statistical differences in mean urinalysis response variables from rats consuming diets containing either 4114 or 4114GLU maize grain compared with rats consuming the 091 control diet (Table 6).

3.2.5. Organ weights

No diet-related organ weight ratio differences were observed in either the 4114 or 4114GLU groups compared with the respective male and female 091 control groups (Table 7). Following gross evaluation and weighing of the total of 288 kidneys that were removed, three were not available for processing into slides (one from a female in the 091 Control group and the other two from a female in the 4114GLU group) though they were weighed and documented as grossly normal at necropsy.

The only statistically significant ($p < 0.05$) organ weight difference was a slightly lower mean absolute (11%) and relative (% brain weight; 10%) epididymides weight in the 4114GLU males when compared with the 091 control group (data not shown). This difference was considered spurious since there was no difference in the mean relative (% body weight) epididymides weights (Table 7), the 091 control group mean absolute epididymides weight was 3–7% higher than each of the reference group mean values, the 4114 group mean values of these variables were not statistically different from the values in the control group, and all mean epididymides weight and relative weight variables were within the range observed in historical control animals (data not shown) and there was no evidence of gross or microscopic changes in the epididymides.

3.2.6. Pathology and histopathology

All but one of the rats survived to the scheduled sacrifice. One female in one of the reference control groups (32D78) was first observed with a mammary gland mass (later determined to be adenocarcinoma) on Day 66 of the study and sacrificed *in extremis* because of the mass on Day 76. The tumor was considered spontaneous.

There were no test diet related gross findings in rats consuming either 4114 or 4114GLU maize grain.

A summary of the microscopic findings is presented in Table 8. A number of histologic observations were noted in this study but were background lesions and representative of what would be expected for rats of this age and strain. Furthermore, they were randomly distributed indicating that there was no evidence of test diet related adverse effects from consumption of diets containing either 4114 or 4114GLU maize grain.

Two males in the 4114 group were diagnosed with bilateral, multiple, renal tubule tumors (RTT) of the amphophilic-vacuolar type (AV) in association with multifocal atypical tubule hyperplasia. In one animal there were 4–5 benign adenomas in each kidney while the other had a benign adenoma in each kidney and a single carcinoma in one kidney. The carcinoma was observed at gross

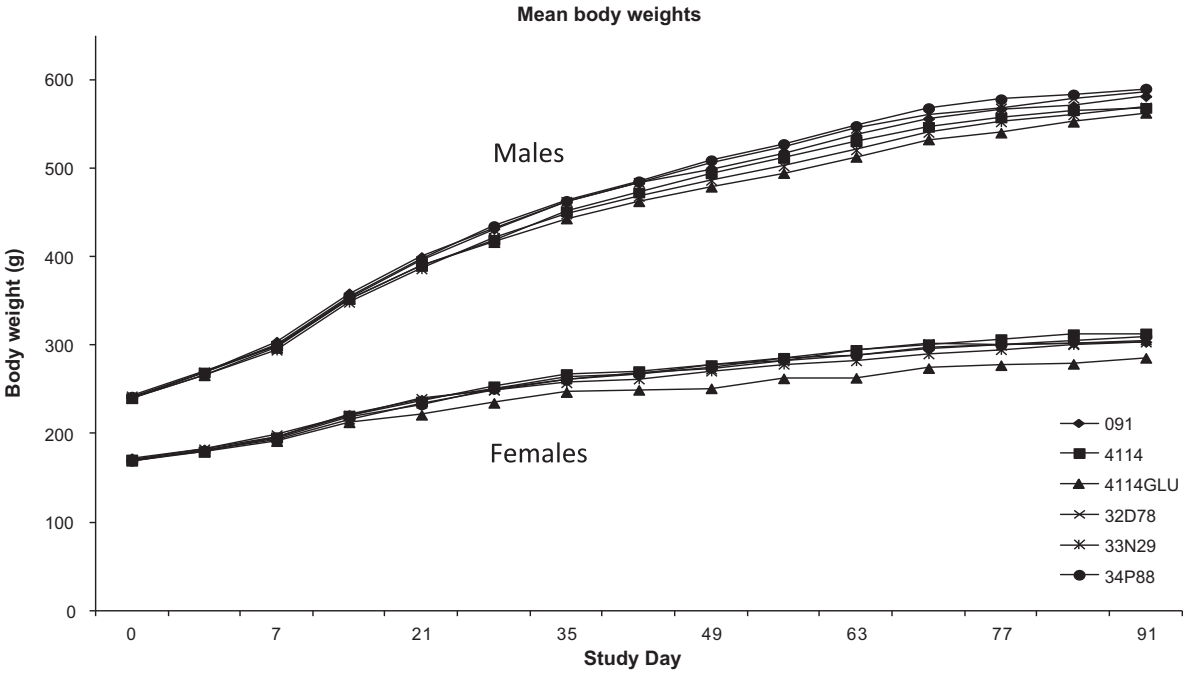

Fig. 1. Mean weekly body weights of male and female rats. Rats were fed experimental rodent diets containing one of six maize grain sources for at least 90 consecutive days. 091, near-isogenic control maize; 4114, genetically modified (GM) maize, 4114GLU GM maize from herbicide-treated plants; 32D78, 33N29, and 354P88, non-GM commercial reference maize.

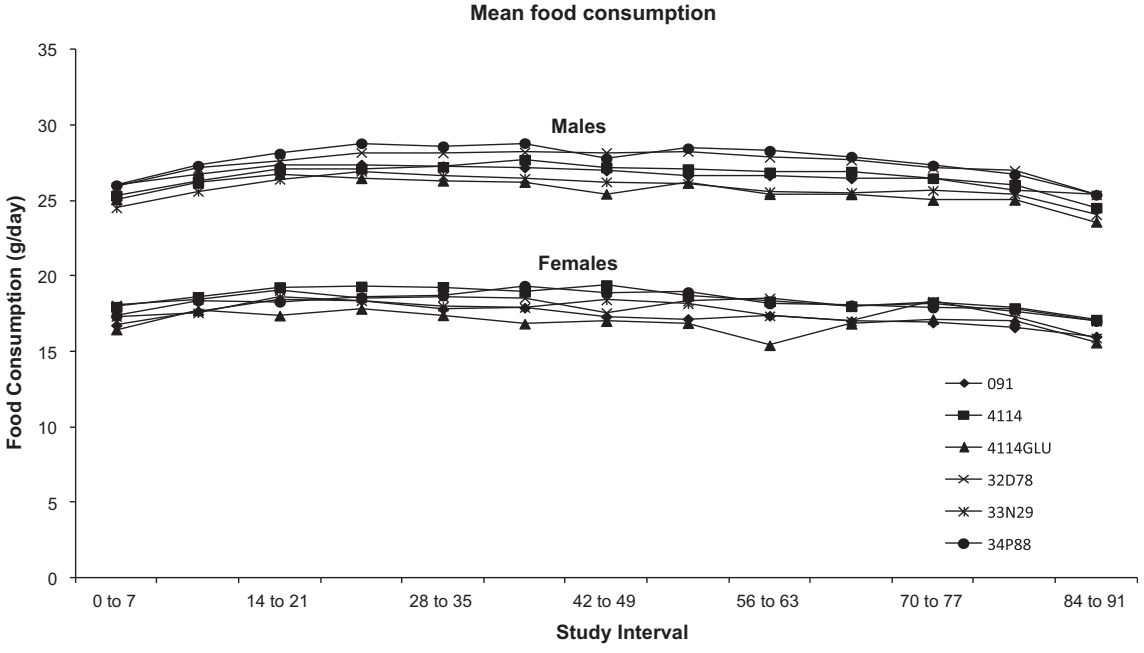

Fig. 2. Mean weekly feed consumption of male and female rats. Rats were fed experimental rodent diets containing one of six maize grain sources for at least 90 consecutive days. 091, near-isogenic control maize; 4114, genetically modified (GM) maize, 4114GLU GM maize from herbicide-treated plants; 32D78, 33N29, and 354P88, non-GM commercial reference maize.

necropsy prior to tissue processing and slide preparation. The diagnosis of carcinoma was based on the presence of large amphophilic and small basophilic cell types, large tumor size, and previous characterization of this morphology as an AV tubule carcinoma. The renal tubule tumors were morphologically typical of those described previously for spontaneous AV tubule adenomas in Sprague–Dawley rats from 90-day toxicity studies (Lanzoni et al., 2007; Hard et al., 1994, 2008). An independent panel of expert pathologists was convened as a Pathology Working Group (PWG) to evaluate the slides of the kidneys from these rats. The PWG determined that the renal tubule tumors were spontaneous and unrelated to consumption of the test diet. The findings of the panel are described below in Section 4 and in greater detail in the accompanying paper (Hardisty et al., submitted for publication).

Table 2
Hematology and coagulation values for male rats (Day 92–93; mean ± SD).

	091 $n = 12$	4114 $n = 12$	4114GLU $n = 12$	32D78 $n = 12$	33N29 $n = 12$	34P88 $n = 12$
RBC ($\times 10^6/\mu L$)	8.61 ± 0.27	8.81 ± 0.31	8.54 ± 0.36	8.88 ± 0.39	8.76 ± 0.27	8.88 ± 0.35
HGB (g/dL)	15.8 ± 0.4	16.3 ± 0.5*	15.8 ± 0.4	16.0 ± 0.5	15.7 ± 0.5	15.9 ± 0.6
HCT (%)	45.8 ± 1.1	47.7 ± 1.7*	46.2 ± 1.5	46.9 ± 1.6	46.0 ± 1.5	46.2 ± 1.9
MCV (fL)	53.3 ± 2.1	53.9 ± 2.0	54.1 ± 1.7	52.8 ± 1.4	52.5 ± 1.8	52.1 ± 1.2
MCH (pg)	18.4 ± 0.8	18.5 ± 0.6	18.6 ± 0.6	18.0 ± 0.6	18.0 ± 0.8	17.9 ± 0.5
MCHC (g/dL)	34.4 ± 0.6	34.3 ± 0.9	34.3 ± 0.9	34.1 ± 0.4	34.2 ± 0.7	34.4 ± 0.5
RDW (%)	12.7 ± 0.5	12.6 ± 0.7	12.9 ± 1.1	12.9 ± 0.7	12.8 ± 0.8	12.5 ± 0.5
ARET ($\times 10^3/\mu L$)	175.53 ± 41.97	164.40 ± 24.52	199.19 ± 72.48	186.52 ± 33.64	159.29 ± 33.47	169.99 ± 52.01
PLT ($\times 10^3/\mu L$)	1079 ± 71	1112 ± 261	1089 ± 76	1046 ± 87	1096 ± 91	1069 ± 95
WBC ($\times 10^3/\mu L$)	9.71 ± 2.50	9.12 ± 1.76	8.56 ± 1.70	10.05 ± 2.47	8.96 ± 2.12	9.36 ± 1.79
ANEU ($\times 10^3/\mu L$)	1.25 ± 0.31	1.20 ± 0.33	1.18 ± 0.38	1.47 ± 0.52	1.29 ± 0.45	1.11 ± 0.33
ALYM ($\times 10^3/\mu L$)	8.00 ± 2.36	7.45 ± 1.66	7.03 ± 1.37	8.12 ± 2.36	7.24 ± 1.92	7.81 ± 1.70
AMON ($\times 10^3/\mu L$)	0.20 ± 0.05	0.22 ± 0.10	0.16 ± 0.04	0.22 ± 0.06	0.22 ± 0.07	0.22 ± 0.06
AEOS ($\times 10^3/\mu L$)	0.16 ± 0.05	0.16 ± 0.08	0.13 ± 0.06	0.16 ± 0.04	0.14 ± 0.05	0.15 ± 0.06
ABAS ($\times 10^3/\mu L$)	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.01
ALUC ($\times 10^3/\mu L$)	0.08 ± 0.04	0.06 ± 0.03	0.05 ± 0.02	0.06 ± 0.03	0.06 ± 0.03	0.05 ± 0.02
PT (s)	11.2 ± 0.9	11.3 ± 0.6	11.2 ± 0.8	11.1 ± 0.4[a]	11.2 ± 0.9	11.1 ± 0.7
APTT (s)	16.3 ± 1.5	16.8 ± 1.6	16.1 ± 1.6	17.1 ± 1.0[a]	16.4 ± 1.4	16.0 ± 1.6

*Statistically significant difference from 091 control at $p < 0.05$ by one-way analysis of variance followed by linear contrasts.
Results are presented as mean ± SD.

Table 3
Hematology and coagulation values for female rats (Day 93–97; mean ± SD).

	091 $n = 10$	4114 $n = 12$	4114GLU $n = 9$	32D78 $n = 10$	33N29 $n = 12$	34P88 $n = 10$
RBC ($x10^6/\mu L$)	8.29 ± 0.32	8.23 ± 0.33	8.23 ± 0.25	8.32 ± 0.32	8.29 ± 0.37	8.32 ± 0.24
HGB (g/dL)	15.7 ± 0.6	15.7 ± 0.4	15.7 ± 0.4	16.0 ± 0.6	15.7 ± 0.5	15.9 ± 0.4
HCT (%)	45.5 ± 1.3	45.2 ± 1.2	44.9 ± 1.3	46.1 ± 1.8	45.2 ± 1.2	45.5 ± 1.2
MCV (fL)	55.0 ± 1.4	55.0 ± 2.5	54.5 ± 1.5	55.5 ± 2.7	54.6 ± 2.1	54.7 ± 1.7
MCH (pg)	19.0 ± 0.4	19.1 ± 0.6	19.1 ± 0.5	19.3 ± 0.7	19.0 ± 0.6	19.1 ± 0.6
MCHC (g/dL)	34.6 ± 0.6	34.7 ± 0.7	34.9 ± 0.5	34.8 ± 0.7	34.8 ± 0.9	34.9 ± 0.5
RDW (%)	11.3 ± 0.3	11.2 ± 0.5	11.2 ± 0.5	11.4 ± 0.3	11.4 ± 0.5	11.1 ± 0.4
ARET ($\times 10^3/\mu L$)	164.77 ± 28.89	136.97 ± 28.44	151.68 ± 44.40	148.05 ± 35.67	163.48 ± 37.12	144.19 ± 23.24
PLT ($\times 10^3/\mu L$)	1038 ± 101	932 ± 85	982 ± 106	944 ± 156	1015 ± 156	992 ± 118
WBC ($\times 10^3/\mu L$)	5.69 ± 1.48	6.35 ± 1.54	7.11 ± 1.59	6.79 ± 1.30	6.67 ± 2.23	6.31 ± 1.31
ANEU ($\times 10^3/\mu L$)	0.72 ± 0.35	0.92 ± 0.40	0.95 ± 0.37	0.82 ± 0.24	0.98 ± 0.38	0.92 ± 0.23
ALYM ($\times 10^3/\mu L$)	4.72 ± 1.25	5.11 ± 1.21	5.87 ± 1.44	5.65 ± 1.37	5.35 ± 1.93	5.08 ± 1.28
AMON ($\times 10^3/\mu L$)	0.12 ± 0.04	0.15 ± 0.09	0.13 ± 0.06	0.16 ± 0.04	0.16 ± 0.06	0.14 ± 0.05
AEOS ($\times 10^3/\mu L$)	0.09 ± 0.04	0.12 ± 0.04	0.10 ± 0.02	0.11 ± 0.04	0.12 ± 0.06	0.11 ± 0.03
ABAS ($\times 10^3/\mu L$)	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
ALUC ($\times 10^3/\mu L$)	0.04 ± 0.01	0.04 ± 0.03	0.06 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.02
PT (s)	10.0 ± 0.3[b]	9.8 ± 0.4	9.9 ± 0.3[a]	10.0 ± 0.2[c]	9.9 ± 0.2	9.9 ± 0.2[a]
APTT (s)	13.9 ± 0.6[b]	14.1 ± 0.9	14.1 ± 0.8[a]	14.2 ± 0.8[c]	13.9 ± 0.6	14.3 ± 0.9[a]

There were no statistically significant differences from 091 Control at $p < 0.05$.
[a]$n = 12$; [b]$n = 11$; [c]$n = 10$; $n < 12$ because the EDTA whole blood sample was clotted and unusable for hematology analyses and whole blood was not available for the rat from the 32D78 group that was sacrificed *in extremis* on Day 76.
Results are presented as means ± SD.

4. Discussion

During the development of GM crops, the transgenic proteins that are to be present within them are subjected to numerous *in silico*, *in vitro*, and in some cases animal studies to determine if they are likely to present risk for allergenicity or toxicity (Codex, 2003; Delaney et al., 2008). 4114 maize is a GM crop developed with a transformation plasmid containing four expressed genes that encode four transgenic proteins: Cry1F, Cry34Ab1, Cry35Ab1, and PAT. As discussed in prior publications, these proteins do not present a risk for allergenicity or toxicity (Herman et al., 2003; Hérouet et al., 2005; Ladics et al., 2006; Delaney et al., 2008; Juberg et al., 2009).

Crops which contain transgenic proteins are also subjected to a comprehensive assessment that focuses on demonstrating that they are "as safe as" conventional crops with a history of safe use (i.e., substantial equivalence; WHO, 1991, 1995). Whole grains and processed fractions from numerous GM crops have also been evaluated in subchronic rodent feeding studies to determine if unintended adverse pleiotropic changes could have occurred as a consequence of genetic modification (Hammond et al., 2006a,b, 2004; MacKenzie et al., 2007; Malley et al., 2007; Appenzeller et al., 2008; He et al., 2008; Healy et al., 2008; reviewed in Delaney, 2007). Of particular relevance to 4114 maize, no test diet related adverse effects were observed in subchronic rodent feeding studies conducted with maize grain from the GM crops in which the same transgenic proteins are expressed (Malley et al., 2007; Appenzeller et al., 2008, 2009).

The results from the current study support the safety of 4114 maize in that no test diet related adverse effects were observed in rats consuming diets containing 4114 maize grain that was or was not treated in the field with glufosinate. As with other 90 day feeding studies with maize grains obtained from GM crops, statistical differences were observed in a number of response variables between the 091 control group and the 4114 and 4114GLU groups, but the differences were spurious and not test diet related

Table 4
Serum chemistry values for male rats (Day 92–93; mean ± SD).

	091 *n* = 12	4114 *n* = 12	4114GLU *n* = 12	32D78 *n* = 12	33N29 *n* = 12	34P88 *n* = 12
AST (U/L)	105 ± 23	97 ± 31	104 ± 31	102 ± 25	102 ± 33	106 ± 24
ALT (U/L)	36 ± 8	34 ± 7	35 ± 3	39 ± 8	46 ± 20	36 ± 7
SDH (U/L)	7.3 ± 4.6	8.9 ± 4.5	8.5 ± 6.8	10.3 ± 6.1	13.8 ± 7.6	7.9 ± 6.0
ALKP (U/L)	80 ± 21	77 ± 18	96 ± 13@	93 ± 21	90 ± 20	82 ± 19
BILI (mg/dL)	013.±0.02	0.12 ± 0.02	0.12 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.12 ± 0.02
BUN (mg/dL)	16 ± 2	16 ± 2	15 ± 2	16 ± 2	15 ± 2	15 ± 1
CREA (mg/dL)	0.37 ± 0.04	0.38 ± 0.04	0.44 ± 0.07*	0.41 ± 0.06	0.41 ± 0.05	0.37 ± 0.05
CHOL (mg/dL)	70 ± 15	68 ± 14	62 ± 11	70 ± 13	65 ± 10	73 ± 19
TRIG (mg/dL)	70 ± 12	69 ± 23	68 ± 23	82 ± 34	66 ± 22	68 ± 19
GLUC (mg/dL)	126 ± 16	125 ± 10	134 ± 16	127 ± 14	135 ± 15	129 ± 12
TP (g/dL)	6.5 ± 0.4	6.6 ± 0.2	6.5 ± 0.3	6.7 ± 0.3	6.7 ± 0.2	6.5 ± 0.2
ALB (g/dL)	3.5 ± 0.2	3.5 ± 0.1	3.5 ± 0.1	3.5 ± 0.2	3.5 ± 0.1	3.4 ± 0.1
GLOB (g/dL)	3.1 ± 0.2	3.1 ± 0.2	3.0 ± 0.3	3.2 ± 0.2	3.2 ± 0.2	3.1 ± 0.2
CALC (mg/dL)	9.8 ± 0.4	9.7 ± 0.2	9.6 ± 0.3	9.9 ± 0.3	9.8 ± 0.2	9.7 ± 0.3
IPHS (mg/dL)	6.6 ± 0.4	6.4 ± 0.8	6.5 ± 0.8	6.8 ± 0.5	6.8 ± 0.7	6.7 ± 0.6
NA (mmol/L)	145.5 ± 5.1	144.5 ± 2.6	144.5 ± 1.8	146.0 ± 4.1	147.0 ± 4.9	146.5 ± 5.3
K (mmol/L)	5.25 ± 0.41	5.35 ± 0.63	5.03 ± 0.65	5.33 ± 0.48	5.31 ± 0.43	5.22 ± 0.45
CL (mmol/L)	107.7 ± 3.1	107.1 ± 1.9	106.9 ± 1.8	107.5 ± 3.0	108.4 ± 3.0	107.9 ± 4.1

*Statistically significant difference from 091 control at $p < 0.05$ by one-way analysis of variance followed by linear contrasts.
@Statistically significant difference from 091 control at $p < 0.05$ by Dunn's Type I test for linear contrasts.
Results are presented as means ± SD.

Table 5
Serum chemistry values for female rats (Day 93–97; mean ± SD).

	091 *n* = 12	4114 *n* = 12	4114GLU *n* = 12	32D78 *n* = 11	33N29 *n* = 12	34P88 *n* = 12
AST (U/L)	117 ± 33[a]	109 ± 22	114 ± 35	104 ± 12	99 ± 13	123 ± 59
ALT (U/L)	42 ± 18[a]	36 ± 9	37 ± 12	34 ± 6	32 ± 6	41 ± 41
SDH (U/L)	13.2 ± 5.5[b]	8.4 ± 5.1	10.5 ± 5.1	9.1 ± 5.1	14.1 ± 7.4	14.3 ± 8.5
ALKP (U/L)	62 ± 17[a]	54 ± 17	59 ± 20	58 ± 18	64 ± 19	52 ± 24
BILI (mg/dL)	0.15 ± 0.02[a]	0.15 ± 0.03	0.15 ± 0.03	0.15 ± 0.03	0.14 ± 0.02	0.16 ± 0.03
BUN (mg/dL)	18 ± 2	17 ± 2	18 ± 2	18 ± 2	18 ± 2	18 ± 2
CREA (mg/dL)	0.49 ± 0.06[a]	0.46 ± 0.04	0.46 ± 0.06	0.46 ± 0.07	0.50 ± 0.05	0.47 ± 0.07
CHOL (mg/dL)	77 ± 15[a]	83 ± 13	79 ± 20	77 ± 10	86 ± 17	82 ± 11
TRIG (mg/dL)	60 ± 20[a]	59 ± 16	59 ± 17	56 ± 11	75 ± 23	63 ± 15
GLUC (mg/dL)	116 ± 15[a]	117 ± 7	121 ± 15	114 ± 11	120 ± 14	121 ± 15
TP (g/dL)	7.0 ± 0.4[a]	7.1 ± 0.4	7.1 ± 0.4	7.2 ± 0.5	7.0 ± 0.4	7.5 ± 0.5
ALB (g/dL)	4.0 ± 0.3[a]	4.0 ± 0.2	4.0 ± 0.2	4.0 ± 0.3	3.9 ± 0.2	4.2 ± 0.3
GLOB (g/dL)	3.0 ± 0.2[a]	3.1 ± 0.3	3.1 ± 0.2	3.2 ± 0.3	3.1 ± 0.3	3.3 ± 0.3
CALC (mg/dL)	10.3 ± 0.3[a]	10.2 ± 0.3	10.1 ± 0.3	10.3 ± 0.3	10.2 ± 0.4	10.4 ± 0.4
IPHS (mg/dL)	5.5 ± 1.3	5.0 ± 0.8	4.8 ± 0.5	5.6 ± 0.5	5.3 ± 0.6	5.2 ± 0.6
NA (mmol/L)	146.2 ± 0.33	142.7 ± 4.5	147.0 ± 6.8	148.9 ± 9.1	144.9 ± 3.8	145.1 ± 5.8
K (mmol/L)	5.09 ± 0.33	4.80 ± 0.27*	4.70 ± 0.52*	5.27 ± 0.32	4.90 ± 0.31	4.77 ± 0.25
CL (mmol/L)	108.5 ± 4.4	107.0 ± 3.4	110.2 ± 5.4	110.4 ± 6.9	107.5 ± 1.9	107.5 ± 4.8

[a]$n = 11$; [b]$n = 10$.
*Statistically significant difference from 091 control at $p < 0.05$ by one-way analysis of variance followed by linear contrasts.
Results are presented as means ± SD.

Table 6
Urinalysis values (mean ± SD).

	091 *n* = 12	4114 *n* = 12	4114GLU *n* = 12	32D78 *n* = 12	33N29 *n* = 12	34P88 *n* = 12
Males						
UVOL (mL)	12.4 ± 11.8	12.2 ± 9.0	11.9 ± 9.4	13.7 ± 9.2	12.1 ± 8.3	11.5 ± 7.9
SG	1.040 ± 0.024	1.032 ± 0.015	1.035 ± 0.018	1.031 ± 0.018	1.033 ± 0.018	1.037 ± 0.020
pH	6.8 ± 0.4	6.8 ± 0.4	6.7 ± 0.3	7.0 ± 0.6	6.7 ± 0.2	6.9 ± 0.4
URO (EU/dL)	0.3 ± 0.2	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.2	0.2 ± 0.0	0.3 ± 0.2
UMTP (mg/dL)	140 ± 83	132 ± 66	144 ± 66	132 ± 89	160 ± 95	147 ± 79
Females						
UVOL (mL)	6.6 ± 4.1	6.4 ± 4.8[a]	3.9 ± 4.1[a]	6.9 ± 3.7[a]	8.1 ± 9.4	5.9 ± 4.3
SG	1.032 ± 0.013	1.036 ± 0.014[a]	1.052 ± 0.032[a]	1.031 ± -0.015[a]	1.034 ± 0.021	1.036 ± 0.024
pH	6.4 ± 0.4	6.4 ± 0.2[a]	6.6 ± 0.4[a]	6.5 ± 0.4[a]	6.5 ± 0.3	6.7 ± 0.5
URO (EU/dL)	0.2 ± 0.0	0.2 ± 0.0[a]	0.2 ± 0.0[a]	0.2 ± 0.0[a]	0.2 ± 0.0	0.2 ± 0.0
UMTP (mg/dL)	30 ± 15	37 ± 17[a]	66 ± 51[a]	30 ± 14[a]	33 ± 22	40 ± 33

[a]$n = 11$.
There were no statistically significant differences from 091 control at $p < 0.05$.
Results are presented as means ± SD.

Table 7
Organ/body weight ratios (mean ± SD).

	091 $n = 12$	4114 $n = 12$	4114GLU $n = 12$	32D78 $n = 12$	33N29 $n = 12$	34P88 $n = 12$
Males						
Body weight (final, g)	552.7 ± 57.0	540.4 ± 35.4	532.5 ± 65.6	557.8 ± 61.5	542.3 ± 49.3	560.6 ± 55.1
Adrenals	0.012 ± 0.002	0.011 ± 0.001	0.012 ± 0.002	0.011 ± 0.001	0.012 ± 0.002	0.011 ± 0.002
Brain	0.387 ± 0.041	0.395 ± 0.024	0.400 ± 0.050	0.378 ± 0.037	0.401 ± 0.038	0.381 ± 0.033
Epididymides	0.292 ± 0.030	0.284 ± 0.030	0.272 ± 0.032	0.272 ± 0.050	0.284 ± 0.021	0.280 ± 0.039
Heart	0.293 ± 0.029	0.285 ± 0.014	0.302 ± 0.029	0.295 ± 0.021	0.290 ± 0.022	0.294 ± 0.027
Kidneys	0.595 ± 0.051	0.586 ± 0.047[a]	0.584 ± 0.055	0.598 ± 0.059	0.592 ± 0.051	0.616 ± 0.058
Liver	2.594 ± 0.293	2.603 ± 0.175	2.540 ± 0.248	2.678 ± 0.190	5.594 ± 0.180	2.605 ± 0.180
Spleen	0.165 ± 0.036	0.154 ± 0.019	0.147 ± 0.018	0.144 ± 0.015	0.145 ± 0.026	0.156 ± 0.024
Testes	0.647 ± 0.082	0.682 ± 0.083	0.664 ± 0.090	0.626 ± 0.050	0.652 ± 0.039	0.657 ± 0.059
Thymus	0.074 ± 0.012	0.067 ± 0.014	0.086 ± 0.014	0.081 ± 0.022	0.077 ± 0.020	0.078 ± 0.015
Females						
Body weight (final, g)	288.3 ± 29.8	291.6 ± 33.6	265.1 ± 17.1	285.0 ± 35.0[a]	286.0 ± 26.6	289.0 ± 25.6
Adrenals	0.023 ± 0.004	0.023 ± 0.004	0.023 ± 0.004	0.026 ± 0.004[a]	0.023 ± 0.004	0.023 ± 0.004
Brain	0.674 ± 0.058	0.655 ± 0.062	0.708 ± 0.047	0.687 ± 0.069[a]	0.672 ± 0.061	0.653 ± 0.065
Heart	0.348 ± 0.025	0.358 ± 0.053	0.367 ± 0.018	0.354 ± 0.037[a]	0.352 ± 0.040	0.367 ± 0.040
Kidneys	0.631 ± 0.059	0.648 ± 0.067	0.681 ± 0.050	0.659 ± 0.071[a]	0.638 ± 0.038	0.636 ± 0.053
Liver	2.483 ± 0.189	2.536 ± 0.175	2.656 ± 0.209	2.569 ± 0.221[a]	2.553 ± 0.201	2.581 ± 0.137
Ovaries	0.049 ± 0.007	0.047 ± 0.010	0.052 ± 0.007	0.057 ± 0.006[a]	0.049 ± 0.008	0.048 ± 0.010
Spleen	0.179 ± 0.026	0.173 ± 0.026	0.195 ± 0.030	0.171 ± 0.014[a]	0.180 ± 0.024	0.180 ± 0.023
Thymus	0.105 ± 0.027	0.115 ± 0.018	0.120 ± 0.024	0.115 ± 0.026[a]	0.112 ± 0.019	0.106 ± 0.023
Uterus	0.273 ± 0.095	0.241 ± 0.072	0.233 ± 0.070	0.235 ± 0.100[a]	0.224 ± 0.066	0.243 ± 0.073

[a] $n = 11$.
There were no statistically significant differences from 091 Control at $p < 0.05$.
Results are presented as means ± SD.

(Hammond et al., 2006a; MacKenzie et al., 2007; Malley et al., 2007; Appenzeller et al., 2008, 2009; Healy et al., 2008).

Two males from the 4114 group were diagnosed with bilateral, multiple, renal tubule tumors (RTT) of the amphophilic-vacuolar type (AV) in association with multifocal atypical tubule hype rplasia. Following the initial observation from the study pathologist, step sectioning of the remaining kidney tissues was conducted for all available rats from the 091 control group, the three reference maize grain groups (32D78, 33N29, and 34P88) as well as the rats from the 4114 and 4114GLU treatment groups as recommended by Eustis and colleagues (1994). Slides produced from the step sectioning confirmed the original findings of AV tumors in the two male rats from the 4114 group but did not note anything other than background lesions in any of the kidneys from any of the other treatment groups (data not shown). A Pathology Working Group was later convened to review the kidney slides from the 091, 4114, and 4114GLU groups and they too confirmed the findings and concluded that these tumors were spontaneous and not related to exposure to the 4114 maize grain. The methods and conclusions of the Pathology Working Group are summarized in a separate paper (Hardisty et al., submitted for publication).

The finding of spontaneous renal tubule tumors in rats has been observed in previous short term studies and in each case the tumors were considered unrelated to treatment (Hard et al., 1994; Hall et al., 2007; Lanzoni et al., 2007). This determination was based on the distinctive histological profile of these tumors that clearly differentiates them from chemically induced renal tumors (Hard et al., 2008). Tubular epithelial cells were enlarged with increased amphophilic cytoplasm, sometimes piling up within the confines of the tubule. The distinction between hyperplastic lesions with papillary projections and small adenomas was sometimes difficult to make as the lesions appeared to represent a proliferative continuum. One renal tubule tumor was diagnosed as a carcinoma based on the presence of both large amphophilic and small basophilic cells, large tumor size, and previous characterization of this morphology as an AV tubule carcinoma (Lanzoni et al., 2007; Hard et al., 2008). There was no evidence of nephrotoxicity in any kidneys in this study, including the kidneys with the atypical tubule hyperplasia and AV tumors. The benign adenomas were morphologically typical of those described previously for spontaneous AV tubule adenomas in Sprague–Dawley rats from 90-day toxicity studies and in longer term rodent feeding studies (Hard et al., 1994, 2008; Hall et al., 2007; Lanzoni et al., 2007).

As noted in Section 3, one animal in a reference group (32D78) was euthanized prior to the scheduled sacrifice because of an ulcerated mammary gland mass which was later diagnosed as a mammary gland adenocarcinoma. Although mammary gland adenocarcinomas are not common in 90-day feeding studies they have been reported to occur spontaneously in animals as young as 10 weeks of age (Oishi et al., 1995) and they are common in older rats (Son, 2004; Son and Gopinath, 2004; Brix et al., 2005). Therefore, this tumor was considered incidental and typical of this neoplasm that occurs naturally in laboratory rats and not related to dietary exposure to 4114 maize grain because the animals in that group consumed diets containing commercially available non-GM maize grain (Giknis and Clifford, 2004).

The renal tubule adenomas and carcinoma and renal tubule hyperplasia observed in the two male rats from the 4114 group were spontaneous lesions unrelated to consumption of the test diet because: (i) they were morphologically identical to specific hyperplastic and neoplastic lesions previously reported to occur spontaneously in multiple rat strains, including Crl:CD(SD)IGS rats; (ii) there are no reports of an increase in AV tubule neoplasms as a result of test material exposure (Hard et al., 2008); (iii) the young age of onset for the tumors in these two rats is typical of spontaneous renal tumors in genetically susceptible rats and not characteristic of chemically-induced renal tumors; and (iv) there was no evidence of cytotoxic changes consistent with pre-neoplastic tubular epithelial cytotoxicity (i.e., nephrotoxicity), as is typical of chemically-induced renal tubular neoplasia. In addition to the morphology of the tumors themselves, the fact that not tumors were observed in the 4114GLU males or in either female treatment group and there were no preneoplastic changes observed in any of the laboratory animals supports the conclusion that the tumors that were observed were not treatment related.

The pre-commercial safety assessment of biotech crops includes an assessment of the transgenic proteins for acute toxicity and similarity to allergenic proteins as well as an evaluation of the

Table 8
Summary of microscopic incidence findings.

Tissue	Finding	Male			Female		
		091	4114	4114GLU	091	4114	4114GLU
Cecum	Inflammation, mucosal	0	0	0	1	1	2
	Minimal	[0]	[0]	[0]	[1]	[0]	[1]
	Mild	[0]	[0]	[0]	[0]	[1]	[1]
Eyes	Degeneration/atrophy, retinal, multifocal	1	0	1	0	0	0
	Minimal	[1]	[0]	[0]	[0]	[0]	[0]
	Mild	[0]	[0]	[1]	[0]	[0]	[0]
	Fold/rosette, retinal, minimal	1	0	1	0	0	0
Heart	Cardiomyopathy, minimal	3	2	2	0	1	1
Kidney	Aggregates, lymphoid	5	5	6	4	4	4
	Minimal	[5]	[5]	[5]	[4]	[4]	[3]
	Mild	[0]	[0]	[1]	[0]	[0]	[1]
	Atrophy, focal tubular, minimal	1	2	0	0	0	2
	Chronic progressive nephropathy, minimal	9	11	8	1	4	3
	Cysts, tubular	1	3	0	1	0	1
	Minimal	[1]	[2]	[0]	[0]	[0]	[1]
	Mild	[0]	[1]	[0]	[1]	[0]	[0]
	Hydronephrosis, unilateral	1	1	1	0	0	0
	Minimal	[0]	[1]	[1]	[0]	[0]	[0]
	Mild	[1]	[0]	[0]	[0]	[0]	[0]
	Hyperplasia, atypical, multifocal, bilateral	0	2	0	0	0	0
	Mild	[0]	[1]	[0]	[0]	[0]	[0]
	Moderate	[0]	[1]	[0]	[0]	[0]	[0]
	Pyelonephritis, unilateral, minimal	0	0	0	0	2	1
	Adenoma, tubular, amphophilic-vacuolar, multiple, bilateral, benign, primary, incidental	0	2	0	0	0	0
	Carcinoma, tubular, amphophilic-vacuolar, multiple, unilateral, malignant without metastasis, primary, incidental	0	1	0	0	0	0
Liver	Fatty change, median cleft	3	1	2	2	3	2
	Minimal	[3]	[1]	[2]	[2]	[1]	[2]
	Mild	[0]	[0]	[0]	[0]	[2]	[0]
	Fatty change, periportal, minimal	1	1	0	1	0	0
	Mononuclear cell infiltrate, minimal	8	8	7	6	9	5
Lungs	Histiocytosis, alveolar, minimal	1	1	1	1	1	0
	Inflammation, perivascular, minimal	0	0	0	1	1	1
Mandibular lymph node	Erythrocytosis/hemosiderosis, sinus	3	1	0	2	0	0
	Minimal	[2]	[0]	[0]	[2]	[0]	[0]
	Mild	[1]	[1]	[0]	[0]	[0]	[0]
	Hyperplasia, lymphoid follicular	1	1	3	2	2	1
	Minimal	[1]	[1]	[1]	[2]	[1]	[1]
	Mild	[0]	[0]	[2]	[0]	[1]	[0]
	Hyperplasia, plasma cell	1	3	0	1	1	1
	Minimal	[1]	[1]	[0]	[1]	[1]	[1]
	Mild	[0]	[2]	[1]	[0]	[0]	[0]
Nose	Inflammation, turbinates, minimal	2	2	1	0	1	0
Pancreas	Atrophy, lobular	2	0	0	0	0	0
	Minimal	[1]	[0]	[0]	[0]	[0]	[0]
	Mild	[1]	[0]	[0]	[0]	[0]	[0]
	Inflammation, multifocal, minimal	1	1	0	0	0	0
Peyer's patch	Mineralization	0	0	0	2	0	0
	Minimal	[0]	[0]	[0]	[1]	[0]	[0]
	Mild	[0]	[0]	[0]	[1]	[0]	[0]
Pharynx	Inflammation	1	1	0	0	0	3
	Minimal	[1]	[1]	[0]	[0]	[0]	[2]
	Mild	[0]	[0]	[0]	[0]	[0]	[1]
Pituitary gland	Cyst, craniopharyngeal, minimal	0	0	0	0	1	1
Prostate	Aggregates, lymphoid	9	7	8	–	–	–
	Minimal	[7]	[5]	[6]	–	–	–
	Mild	[2]	[2]	[2]	–	–	–
Teeth	Inflammation, periodontal	4	3	6	4	5	9
	Minimal	[2]	[3]	[4]	[4]	[5]	[9]
	Mild	[2]	[0]	[2]	[0]	[0]	[0]
Thymus	Hyperplasia, epithelial, minimal	1	0	0	0	2	2
Thyroid	Cysts, follicular	1	2	0	0	0	0
	Minimal	[1]	[0]	[0]	–	–	–
	Mild	[0]	[2]	[0]	–	–	–
	Cysts, ultimobranchial	2	1	1	1	5	0
	Hypertrophy, follicular cell, minimal	1	2	2	0	0	0
Uterus	Dilatation	–	–	–	7	3	2
	Minimal	–	–	–	[0]	[0]	[1]
	Mild	–	–	–	[6]	[3]	[1]
	Moderate	–	–	–	[1]	[0]	[0]

Table reports microscopic findings where incidence was greater than 1 per treatment group in either the 091, 4114, or 4114GLU treatment groups from a total of 51 tissues examined in each treatment group. The grading (e.g., minimal, mild) of the finding varied, the incidence of each grade is presented in brackets.

genetically-modified crop for unintended changes (i.e., pleiotropic effects) by compositional analyses and performance in animal feeding studies. In this study the nutritional performance of biotech maize grain as compared to conventional maize grain was examined and the 4114 maize grain was evaluated for possible unintended changes in dietary toxicity.

5. Conclusions

The results reported in this paper support the conclusion that grain from 4114 maize is as safe and nutritious as maize grain from conventional maize crops.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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