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Evaluation of Bt (*Bacillus thuringiensis*) Corn on Mouse Testicular Development by Dual Parameter Flow Cytometry

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The health safety of Bt (*Bacillus thuringiensis*) corn (*Zea mays* L.) was studied using mouse testes as a sensitive biomonitor of potential toxic effects. Pregnant mice were fed a Bt corn or a nontransgenic (conventional) diet during gestation and lactation. After they were weaned, young male mice were maintained on the respective diets. At 8, 16, 26, 32, 63, and 87 days after birth, three male mice and an adult reference mouse were killed, the testes were surgically removed, and the percentage of germ cell populations was measured by flow cytometry. Multigenerational studies were conducted in the same manner. There were no apparent differences in percentages of testicular cell populations (haploid, diploid, and tetraploid) between the mice fed the Bt corn diet and those fed the conventional diet. Because of the high rate of cell proliferation and extensive differentiation that makes testicular germ cells highly susceptible to some toxic agents, it was concluded that the Bt corn diet had no measurable or observable effect on fetal, postnatal, pubertal, or adult testicular development. If data from this study were extrapolated to humans, Bt corn is not harmful to human reproductive development.

KEYWORDS: Transgenic crops; Bt corn; *Bacillus thuringiensis*; testicular cell populations; flow cytometry; acridine orange

INTRODUCTION

Transgenic crops are moving to the forefront of agricultural practices in many parts of the world due to the positive benefits of intrinsic pest control and simplified, more environmentally sound weed control. Public concerns about the value and safety of transgenic crops temper this burgeoning biotechnology by demanding that scientific research include possible environmental and health impacts. *Bacillus thuringiensis* (Bt) is a microbe that produces an insecticidal endotoxin and has been used commercially as a topical pesticide since 1961 (*1*). Genetic transformation techniques have allowed the gene expressing these δ-endotoxins that accumulate to form crystalline proteins (Cry proteins; *2*) to be inserted into crop plants. The gene products are expressed primarily in green tissue of the plant but can also be expressed in varying degrees in seeds and pollen. The expressed Cry protein has been shown to reduce European corn borer (*Ostrinia nubilalis* Hubner) stalk tunneling in corn (*Zea mays* L.) by 88−100% (*3*), to reduce but not eliminate stalk borer (*Papaipema nebris* Guenée) damage (*4*), and to provide excellent control of *O. nubilalis* and very good control of corn earworm (*Helicoverpa zea* Boddie) in sweet corn hybrids (*5*). The coexpression of two Bt δ-endotoxins has demonstrated protection from root damage by western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in field tests of corn (*6*) showing that there are lepidopteran specific Bt toxins for corn borers and coleopteran specific Bt toxins for corn rootworm control. The economic and logistic advantages of Bt crop plants, especially for those under high pest pressure, include reduction or elimination of insecticidal spraying and alleviate the difficulty that conventional chemical sprays had in reaching stalk-boring pests (due to boring behavior) and crucial timing of spraying.

Concerns were raised regarding the safety of transgenic crops after two studies were made public by the popular press. One concluded that transgenic potatoes were toxic to rats and compromised their immune systems (*7*) and the other found that Bt corn pollen was hazardous to the monarch butterfly [*Danaus plexippus* L. (*8*)]. Apprehension about transgenic damage to nontarget organisms was fueled as yet another study reported lethal effects of Bt corn pollen on the monarch butterfly (*9*). A series of studies that were published in five PNAS papers in late 2001 indicated that the risk to monarch posed by exposure to Bt was minimal (*10*−*14*).

Feeding trials of event 176 Bt corn in broiler chickens showed no adverse effects on survival, body weights, feed conversion, and breast muscle yield as compared to diets prepared with nontransgenic corn (*15*). Milk production, ruminal pH, and acetate:propionate ratios in dairy cows and daily weight gain and feed efficiency in steer calves and dairy cows were not affected by the incorporation of the Bt trait in the corn (*16*). Additionally, this study found no differences in grazing prefer-

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Table 1. Chemical Composition[a] of Modified Certified Rodent Diet 5002 with Conventional Corn and Soybean Diet (C) and Transgenic Corn Diet (T)

	C	T		C	T
			nutrients[b]		
protein (%)	**21.0**	**21.0**	**fat (acid hydrolysis) (%)**	**3.2**	**3.1**
arginine (%)	1.23	1.24	cholesterol (ppm)	153	153
cystine (%)	0.29	0.28	linoleic acid (%)	12.41	12.78
glycine (%)	0.93	0.93	linolenic acid (%)	0.07	0.07
histidine (%)	0.53	0.53	arachidonic acid (%)	0.01	0.01
isoleucine (%)	1.10	1.11	omega-3 fatty acids (%)	0.20	0.20
leucine (%)	1.68	1.67	total saturated fatty acids (%)	0.59	0.59
lysine (%)	1.26	1.28	total monounsaturated fatty acids (%)	0.66	0.66
methionine (%)	0.43	0.43	**fiber (max) (%)**	**5.1**	**5.2**
phenylalanine (%)	0.98	0.98	neutral detergent fiber[c] (%)	15.3	15.3
tyrosine (%)	0.64	0.64	acid detergent fiber[d] (%)	7.2	7.3
threonine (%)	0.83	0.83	**nitrogen free extract (difference) (%)**	**51.7**	**51.6**
tryptophan (%)	0.25	0.25	starch (%)	29.13	28.63
valine (%)	1.11	1.11	glucose (%)	0.20	0.20
servine (%)	0.61	0.61	fructose (%)	0.25	0.24
aspartic acid (%)	1.30	1.29	sucrose (%)	2.59	2.59
glutamic acid (%)	2.78	2.77	lactose (%)	1.34	1.34
alanine (%)	0.88	0.88	**total digestible nutrients (%)**	**77.6**	**75.2**
proline (%)	1.05	1.05	**gross energy (kcal/g)**	**4.11**	**4.10**
taurine (%)	0.02	0.02	**physiological fuel value[e] (kcal/g)**	**3.47**	**3.46**
fat (ether extract) (%)	**6.2**	**6.2**	**metabolizable energy (kcal/g)**	**3.19**	**3.09**
			minerals		
ash (%)	**6.0**	**6.0**	fluorine (ppm)	6.7	6.6
calcium (%)	0.92	0.92	iron (ppm)	205	204
phosphorus (%)	0.60	0.60	zinc (ppm)	74	74
phosphorus (nonphytate) (%)	0.36	0.38	manganese (ppm)	69	69
potassium (%)	0.99	1.00	copper (ppm)	12	12
magnesium (%)	0.20	0.20	cobalt (ppm)	0.80	0.80
sulfur (%)	0.21	0.21	iodine (ppm)	0.96	0.96
sodium (%)	0.30	0.30	chromium (ppm)	2.48	2.48
chlorine (%)	0.54	0.54	selenium (ppm)	0.24	0.24
			vitamins		
carotene (ppm)	3.7	3.6	pyridoxine (ppm)	7.32	7.32
vitamin K (menadione) (ppm)	0.4	0.4	biotin (ppm)	0.1	0.1
thiamine hydrochloride (ppm)	15	15	vitamin B-12 (mcg/kg)	20	20
riboflavin (ppm)	8.0	8.0	vitamin A (IU/g)	18	18
niacin (ppm)	90	90	vitamin D-3 (added) (IU/g)	2.2	2.2
pantothenic acid (ppm)	18	18	vitamin E (IU/kg)	71	70
choline chloride (ppm)	1800	1800	ascorbic acid (ppm)	0.0	0.0
folic acid (ppm)	3.0	3.0			
			calories provided by		
protein (%)	**24.241**	**24.266**	**carbohydrates (%)**	**59.650**	**59.629**
fat (ether extract) (%)	**16.109**	**16.106**			

[a] On the basis of the latest ingredient analysis information. Because nutrient composition of natural ingredients varies, analysis will also differ accordingly. [b] Expressed as % of ration except where indicated; moisture content is assumed to be 10.0% for the purpose of calculations. [c] NDF = approximately cellulose, hemicellulose, and lignin. [d] ADF = approximately cellulose and lignin. [e] Physiological fuel value (kcal/gm) – sum of decimal fractions of protein, fat, and carbohydrate (nitrogen free extract) × 4, 9, and 4 kcal/gm, respectively.

ence or daily rate of gain in steers grazing Bt vs conventional corn residues. A recent article written by EPA scientists, regulatory staff, and an attorney summarizes the EPA's reassessment finding of no unreasonable adverse effects to human health or the environment, which strengthens the argument for Bt crop safety (*17*).

The objective of this study was to determine whether Bt corn is safe when consumed by mice during pregnancy, early development, and growth into adulthood. Germ cell populations were measured at appropriate intervals during development in mice on a diet containing Bt, including those exposed over three previous generations, to carefully compare testis development with mice on a conventional corn diet. If Cry protein in Bt corn is a toxicant, the high rate of cell proliferation and the unique cellular differentiation in the mammalian testis make it a very sensitive organ that can determine cellular and molecular changes that occur when exposed to transgenic corn (*18*). Our hypothesis was that Bt corn would have no short- or long-term negative effects on mouse fetal, postnatal, pubertal, and adult testicular development.

MATERIALS AND METHODS

Short-Term Mouse Study. *Feed.* Corn grain was obtained in October 2000, from a northeastern South Dakota Bt cornfield and an isolated isogenic cornfield. The Bt corn (38PO6) was taken directly from the combine. The nontransgenic corn (38PO5) was hand picked and shelled with a hand sheller to minimize the probability of contamination with the Bt corn. The South Dakota State University Chemistry Analytical Lab analyzed the grain samples for crude protein, crude fiber, crude fat (ether extract), Ca^{+}, and K^{+}. Rations made with Bt corn and conventional soybeans (*Glycine max* L.) and corn were formulated by Purina Test Diet (Richmond, IN). The chemical composition of each ration is shown in **Table 1**. The transgenic corn ration contained 28.5% Bt corn by weight.

Experimental Design. Bt or conventional corn diets were fed to 20 randomly selected female mice (10 for each diet). Following breeding, gestation, and parturition, three male progeny of the same age (by day)

were chosen at random for each of six time points, 8, 16, 26, 32, 63, and 87 days after birth. Time points were selected to show the various stages of cellular proliferation and differentiation that occur during the development of the testes and ongoing cycles of spermatogenesis. The relative percent of testicular cell populations reflects the kinetics of the developing testes. At 8 days of age, mice should be at the end of the spermatogonial proliferation stage (*19*). The majority of the cells should be in the diploid state as the spermatogonia evolve to primary spermatocytes during mitotic activity. Sixteen day old mice were selected to reflect the meiotic activity of the primary spermatocytes (2*n*) as they develop into haploid spermatids (1*n*). Spermiogenesis initially occurs from 18 to 30 days after birth, and significant temporal changes occur in the cell populations as round spermatids are biochemically and morphologically transformed to elongated spermatids (*19*). Mice are considered to have reached adult stages by 48 days of age.

Three mice were killed for each time point, two subsamples of each testicular suspension were measured by flow cytometry, and 5000 cells of each sample were measured and analyzed. Therefore, for each time point, a total of 30 000 testicular cells were measured and analyzed.

Experimental Animals. Five week old C57BL/6J female and C3H/HeJ male breeding mice were obtained from Jackson Laboratories (Bar Harbor, ME). These two strains of mice were used so that the F1 progeny were of the cross-bred strain used in previous studies (*18*−*24*). The mice were allowed to acclimate to the housing conditions for a 3 week period during which they were fed Purina Mouse Chow 5002. The mice were maintained on their respective diets and deionized water and were housed at 22 ± 2 °C on a 12 h light/dark schedule. At the time of breeding, the specially formulated transgenic or conventional diets were started and fed through gestation and lactation. After they were weaned, the young male mice were continued on the diets of their respective dams. Adult reference mice (F1) were maintained on Purina Mouse Chow 5002 and used to ensure standardization of the flow cytometer and reagents. The experiment was approved and monitored by the Institutional Animal Care and Usage Committee of South Dakota State University.

Tissue Preparation. At 8, 16, 26, 32, 63, and 87 days after birth, three male mice and an adult reference mouse were weighed and killed by cervical dislocation, and the testes were surgically removed. Note that progeny born within the same 24 h time period would be considered the same age. Because of the rapid changes occurring in the testicular development, cell types could show differences based entirely on this 24 h spread. Also, because of instrument limitations, some animals were measured in the morning and others in the afternoon, adding another 8 h of variation. Testes were placed in 60 mm Petri dishes containing 1−2 mL of HBSS (Hank's balanced salt solution), minced with curved surgical scissors, and placed into conical tubes (12 mm × 75 mm). Tissue fragments were allowed to settle, and the supernatant was gravity filtered through 53 μm Nitex filters. All tissues were kept on crushed ice (4 °C) from dissection until measurement by flow cytometry.

Cell Staining and Flow Cytometry. Dual parameter flow cytometry was used to characterize testicular cell populations. Cells were stained with acridine orange, a metachromatic dye that intercalates into double-stranded nucleic acid and produces green fluorescence when excited by blue (488 nm) laser light. Acridine orange interaction with single-stranded DNA or RNA exhibits red fluorescence with the same excitation (*25*). The relative content of red and green fluorescence was used to identify the haploid (1*n*), diploid (2*n*), and tetraploid (4*n*) cell types in the testicular cells (*26*).

Two hundred microliter aliquots of each testicular cell suspension were mixed with 400 μL of an acid−detergent solution containing 0.08 N HCl, 0.15 M NaCl, and 0.1% Triton X-100 (pH 1.2). This caused permeabilization of the cell membrane and pH-induced histone dissociation from testicular cells, which in turn allowed the acridine orange stain access to the DNA (*27*). Thirty seconds later, 1.2 mL of the staining solution containing 0.20 M Na_2HPO_4, 0.1 M citric acid buffer (pH 6.0), 1 mM EDTA, 0.15 M NaCl, and 6.0 μg/mL chromatographically purified acridine orange (Polysciences, Inc., Warrington, PA) was added to the sample (*18*, *28*).

Immediately after staining, approximately 70 cells/s were passed through the quartz flow channel of a flow cytometer interfaced to a PC Cicero Unit (Cytomation, Fort Collins, CO). Recorded measurements were begun 3 min after staining, and fluorescence data were collected on 5000 cells/sample. Red ($F_{\geq 630}$) and green fluorescence ($F_{515-530}$) emitted from each individual cell were separated optically, and the digitized signals were processed and recorded on the computer. Testicular list mode files were analyzed using the Cyclops PC computer program.

Statistical Analysis. Data were statistically analyzed using the General Linear Model procedure of the Statistical Analysis System (SAS for Windows, v. 6.12, SAS Institute Inc., Cary, NC). Homogenicity of variance test showed that variance in day by treatment was not equal; therefore, the Mixed Procedure with Scheffe adjustment was used to analyze the testicular population data. *P* values less than 0.05 were considered significant.

Multigenerational Mouse Study. *Feed.* Corn harvest and preparation and ration formulation followed the same procedure as for the short-term study. Extra feed for the long-term study was stored in airtight bags and kept refrigerated (−20 °C).

Experimental Design. Transgenic or conventional diets were fed to 16 randomly selected male and female mice (two of each sex and strain for each diet). Following breeding, gestation, parturition, and weaning, six female and three male 2nd generation progeny of each strain and diet were randomly selected for growth, development, and breeding to obtain the 3rd generation. Surplus mice from each generation were culled. When all 3rd generation mice were at least 6 weeks old, six C57Bl/6J females and three C3H/HeJ males were randomly chosen and bred to obtain 4th generation cross-bred progeny. Three male progeny of the same age (by day) were chosen at random for each of five time points. Time points and sampling followed the same procedure as the subchronic study, except that the extended adult time point at 87 days postpartum was deleted.

Experimental Animals. Five week old C3H/HeJ males and females and C57Bl/6J males and females were obtained from Jackson Laboratories, allowed to acclimate to the facilities, and bred. Care of the mice followed the procedure above. Three generations of the respective lines were fed the Bt corn or the conventional corn diet. The fourth generation was a cross between C57Bl/6J females and C3H/HeJ males of each diet in order to maintain the use of the cross-bred animal as the measured population as in the short-term study above and previous toxicology studies on mice (*18*, *20*, *22*−*24*).

Tissue Preparation. At 8, 16, 26, 32, and 63 days after birth, three male mice and an adult reference mouse were weighed and killed by cervical dislocation, and the testes were surgically removed. Tissue preparation for flow cytometry followed the same procedure as above in the short-term study.

Cell Staining and Flow Cytometry. Cell staining and flow cytometry followed the same procedure as above in the short-term study.

Statistical Analysis. Statistical analysis followed the same procedure as above in the short-term study.

Positive Control. Positive control F1 (C57BL/6J × C3H/HeJ) males were injected at 26 days after birth and for five consecutive days with 0.2 mL of phosphate-buffered saline containing 300 mg/kg of body weight of hydroxyurea (Sigma Chemical Co., St. Louis, MO). Three mice were killed 6 days after the last injection of hydroxyurea, and the testicular cells were measured by flow cytometry as described above.

RESULTS

Short-Term Mouse Study. *Testicular Cell Kinetics.* The relative percent of testicular cell populations reflects the kinetics of the developing testes. Near the end of the spermatogonial proliferation state at 8 days of age, the majority of the cells are in the diploid stage (90.2% ± 1.0, transgenic Bt; 88.4% ± 1.4, control) as the spermatogonia evolve to primary spermatocytes during mitotic activity. Cytograms from 8 day old mice on conventional and Bt corn diets show the diploid, tetraploid, and active S phase populations (**Figure 1A**,**a**, respectively). For

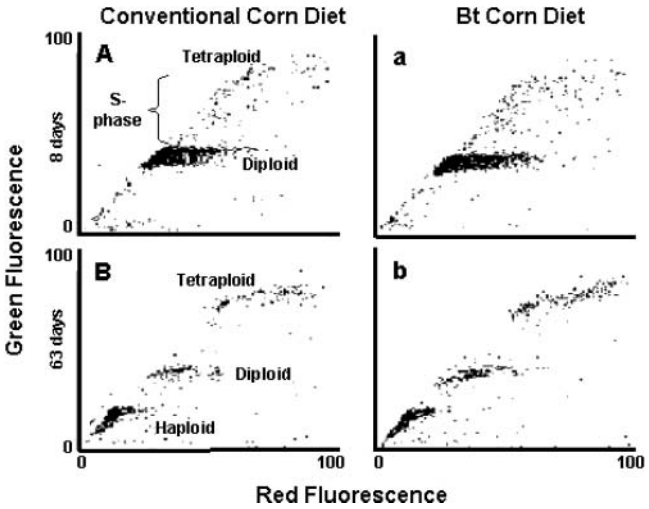

Figure 1. Green vs red fluorescence cytograms of 2000 acridine orange stained testicular cells from 8 day old mice fed (**A**) a conventional corn-fed and (**a**) a Bt corn diet. Most cells at this stage of growth are diploid (center population). Cytograms **B** and **b** are from 63 day old mice of the 4th generation of the multigenerational study. Haploid cells comprise approximately 80% of all testicular cells in both the conventional and the Bt diets. The 8 and 63 day old mice were presented together so that the reader could visualize the typical adult testicular profile after measuring the cells as described above.

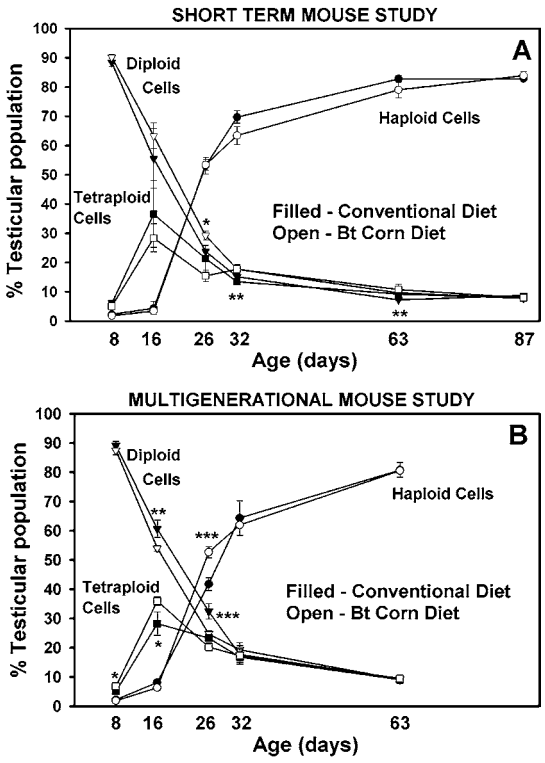

Figure 2. (**A**) Percentages (mean ± SD) of tetraploid, diploid, and haploid testicular populations of conventional and Bt corn-fed mice over the time course of the short-term mouse study. There were three mice per treatment per time point. (**B**) Progression of testicular populations from the 4th generation of the multigenerational study from days 8 to 63. Means ± SD ($n = 3$) are shown for both the conventional and the Bt corn diets. Variations seen at 16 and 26 days of age were likely related to small age differences (≤32 h) of the mice measured. Data from the short-term and multigenerational studies are presented together so that the reader may see how precise these measurements are. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; $n = 3$ for each diet or treatment.

comparison, **Figure 2A** gives relative percentages of testicular cell types measured for all days between the Bt and the conventional corn diets. Flow cytometric measurements at 16 days showed a relative decrease in diploid cells (Bt = 63.3% ± 4.4; conventional = 55.5% ± 10.2) and a relative increase in tetraploid cells (Bt = 28.4% ± 4.8; conventional = 36.6% ± 11.5) reflecting the meiotic activity of the primary spermatocytes (2*n*) as they develop into haploid spermatids (1*n*). Spermiogenesis initially occurs from 18 to 30 days after birth when round spermatids are biochemically and morphologically transformed to elongated spermatids (*19*). The rapid rate of spermiogenesis is demonstrated by the large standard deviations (SD) during those time periods, particularly in the tetraploid population (**Figure 2A**).

At 26 days, the average percentages of haploid, diploid, and tetraploid populations for the Bt corn-fed animals were 53.4 ± 1.8, 29.2 ± 1.5, and 15.4 ± 1.9, respectively, and 53.0 ± 2.8, 23.8 ± 2.2, and 21.3 ± 4.5, respectively, for the conventional corn-fed animals. At 32 days of age, the relative percent of cell populations becomes more stable, although a difference of up to 32 h from birth to sample measurement occurred between the conventional and the Bt corn-fed animals. No differences between conventional and Bt corn-fed mice were seen at 87 days after birth as ongoing cycles of spermatogenesis ensued.

General Health Parameters. Mice fed the Bt corn diet showed no differences in body weight through day 63 ($P = 0.1074$) as compared to those on the conventional corn diet (**Figure 3A**) suggesting that the Bt corn diet caused no negative impact on body growth. However, the 87 day old conventional diet mice were fatter. Note that the 63 day old mice were the exact opposite (Bt corn diet weighed more), and this was attributed to individual eating behavior after adulthood is achieved. Average litter sizes in the two groups were similar (Bt = 7.2 ± 1.0, $n = 9$; conventional = 7.3 ± 1.0, $n = 9$).

Multigenerational Mouse Study. *Testicular Cell Kinetics.* Cytograms of 63 day old, 4th generation mice fed conventional and Bt corn diet depict the distribution of the adult testicular cell populations (**Figure 1B**,**b**). The Bt corn-fed mice averaged 80.6 ± 0.8, 9.2 ± 0.8, and 9.5 ± 0.3%, respectively, for haploid, diploid, and tetraploid populations while the conventional corn-fed mice averaged 80.8 ± 2.5, 9.3 ± 1.5, and 9.2 ± 1.1%, respectively. As **Figure 2B** illustrates, the feeding of Bt corn to mice for four generations caused no differences in the testicular populations at 32 or 63 days of age. The slight differences between the conventional and the Bt corn-fed mice seen at other time points are likely due to the differences in timing of birth, as discussed above.

General Health Parameters. Mean body weights showed differences between conventional and transgenic corn-fed mice at 26 ($P < 0.0001$) and 63 ($P = 0.0100$) days (**Figure 3B**). At both time periods, the Bt corn-fed mice were heavier than the control mice. Mean body weight results suggest that ingestion of a Bt corn diet resulted in no multigenerational impacts on animal growth. Average litter sizes were comparable (Bt = 8.2 ± 1.9, $n = 5$; conventional = 6.6 ± 2.1, $n = 5$).

Positive Control. Hydroxyurea was used as a positive control and showed a reduction in certain testicular populations due to exposure. The dual parameter cytograms (**Figure 4**) generated from conventional corn-fed mice (**A**) and hydroxyurea-injected mice (**B**) show that the S phase and tetraploid (upper right quadrant) populations were depleted due to hydroxyurea treatment. **Figure 4C** shows the percentages of the major populations for conventional, Bt corn-fed, and hydroxyurea-injected mice at 32 days. The percentage diploid cell population in the

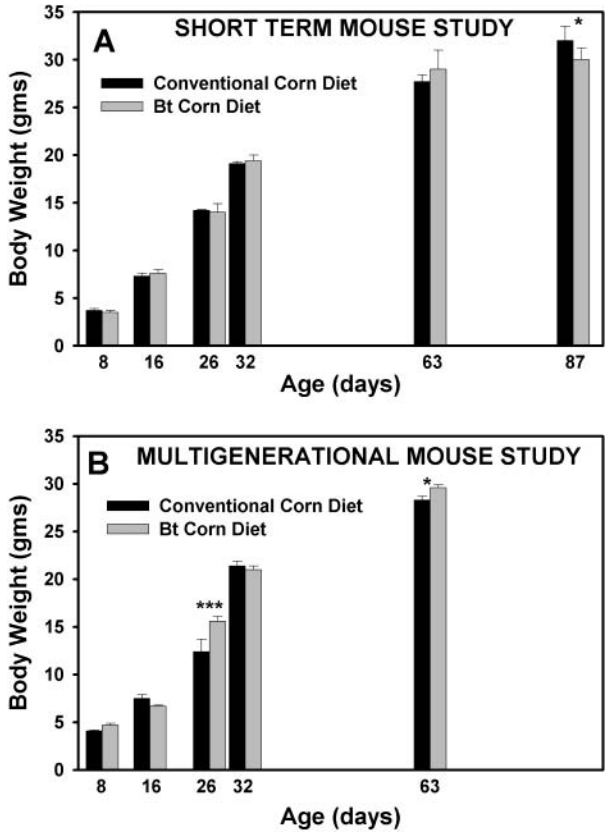

Figure 3. Comparison of body weights between mice fed the conventional or Bt corn diet at the time points measured during the short-term (**A**) and multigenerational (**B**) mouse studies. Data from the short-term and multigenerational studies are presented together so that the reader may see how precise these measurements are. *$P < 0.05$; ***$P < 0.001$; $n = 3$ for each diet or treatment.

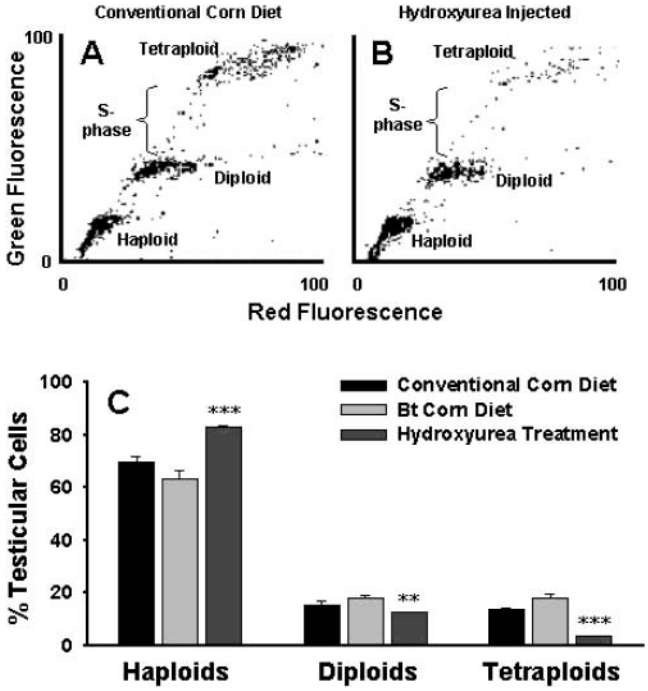

Figure 4. Green vs red fluorescence cytograms of 2000 acridine orange stained testicular cells from a 32 day old mouse on a (**A**) conventional corn diet and (**B**) 36 day old mouse injected with 300 mg/kg hydroxyurea mouse. Note the depleted tetraploid population in the upper right quadrant of cytogram **B**. The testicular cell populations for conventional corn, Bt corn, and hydroxyurea-treated mice are graphed in **C** for comparison. **$P < 0.01$; ***$P < 0.001$; $n = 3$ for each diet or treatment.

hydroxyurea mice is less ($P = 0.0056$) than the conventional corn and Bt corn mice. The hydroxyurea mice showed a decreased tetraploid population ($P < 0.0001$) and a concurrent increase in percent haploid cells ($P < 0.0001$) relative to the mice fed the conventional corn diet.

DISCUSSION

Health safety of Bt corn use in diets was studied using mammalian testes as a sensitive biomonitor of potential toxic effects. DNA synthesis, cellular differentiation, and other molecular functions are involved in the complex development of the testes and ongoing cycles of spermatogenesis. The administration of hydroxyurea or other chemical or environmental assaults can disrupt this well-defined mitotic and meiotic circuit. Use of hydroxyurea-injected mice as a positive control demonstrated that testicular cells can depict abnormal cellular and molecular changes. Hydroxyurea inhibits DNA synthesis by inactivating ribonucleotide reductase (*29*). This well-absorbed, rapidly cleared metabolic inhibitor reduces the tetraploid population of testicular cells and subsequent haploid cells by its stage specific and dose-dependent effects (*30*). Other reproductive toxicants and environmental stressors have shown altered testicular cell profiles when stained with acridine orange and measured by flow cytometry (*18*, *20*−*24*).

Litter sizes are an important parameter in toxicology studies where DNA damage can cause embryo death and resorption in the uterus (*24*, *30*). In the multigenerational study, diet exposure occurred preconceptually so that litter size could reflect effects on fertility (sperm function), DNA/chromosome damage in sperm, or effects on fetal development since the pregnant dams were dosed. However, litter sizes were similar in both short- and long-term studies in the Bt and conventional corn-fed mice, suggesting that the Bt diet is not a developmental toxicant.

This study along with previous studies by Janca et al. (*19*) and Brake and Evenson (*31*) have shown that cellular changes in the testes during the first round of meiosis and spermiogenesis occur very rapidly, where several hours difference in age can make a significant difference in the percentages of cellular populations. However, the percent haploid cells, the ultimate product of spermatogenesis, are similar by 32 days on both diets and protocols (short-term and multigenerational). The differences in the percentages of diploid and tetraploid cells, although significant, are minor and do not translate into significant changes in haploid cells. Beyond this extremely variable time period of normal developmental spermatogenesis, this study has shown that a diet of Bt corn caused no differences in the percentages of haploid, diploid, and tetraploid testicular cell populations in short- (subchronic) and long-term studies. Ingestion of Bt corn in a nutritionally balanced diet by the mother during pregnancy and lactation and later by the young developing male mouse had no negative effect on fetal, postnatal, pubertal, or adult testicular development or body growth.

ABBREVIATIONS USED

Bt, *Bacillus thuringiensis*; Cry proteins, crystalline proteins; HBSS, Hank's balanced salt solution.

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