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A 90-day feeding study of glyphosate-tolerant maize with the *G2-aroA* gene in Sprague-Dawley rats

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ABSTRACT

Maize is not only a staple food crop but also an important raw material for feed and industry; however, the threat of weeds leads to a serious decline in its output and quality. The *G2-aroA* gene confers glyphosate herbicide tolerance to crops. In this study, the food safety of genetically modified (GM), glyphosate-tolerant maize with the *G2-aroA* gene was evaluated in a 90-day feeding study in Sprague-Dawley (SD) rats. Maize grain from GM or non-GM isogenic control lines were separately formulated into rodent diets at concentrations of 12.5% (low level), 25% (middle level), and 50% (high level). An additional group of rats were fed a commercialized diet as a control. The toxicological response variables, including body weights, food consumption, serum biochemistry, hematology, and absolute and relative organ weights, were compared between rats fed GM maize and those fed non-GM maize after consumption of test diets for 90 days. In addition, gross and microscopic pathology were conducted among treatment groups. No adverse effects related to the consumption of GM maize were detected in the subchronic feeding study. These results indicated that the GM glyphosate-tolerant maize was as safe and nutritious as conventional maize.

1. Introduction

Maize is not only a staple food crop but also an important raw material for feed and industry because it is nutritious, and historically, has been inexpensive. Nevertheless, the threat of weeds leads to a serious decline in its output and quality. The birth of herbicides provided an effective means for the prevention and treatment of weeds. Historically, there are non-transgenic herbicide resistance (HR) traits for cyclohexanedione herbicides, imidazolinone herbicides, sulfonylurea herbicides and triazine herbicides. However, most of the current commercialized herbicide- resistant crops are cultivars created by genetic modification (Duke, 2005). In 2011, genetically modified (GM) crops are cultivated at 160 million hectares around the world, approximately 59% of which were herbicide resistant crops (James, 2012).

The mode-of-action of herbicide resistance is facilitated by the insertion of five different genes to confer resistance to the respective herbicides: the glyphosate oxidoreductase (*GOX*) gene or mutant 5-enolphyruvylshikimate-3-phosphate synthase gene (*EPSPS*) for glyphosate resistance (such as *CP4-EPSPS* isolated from an *Agrobacterium* strain *CP4*), the nitrilase gene for bromoxynil resistance and the *bar* gene for glufosinate resistance (Duke, 2005). In recent years, two novel genes, *gat4621* and *hra*, were introduced which conferred high levels of resistance to glyphosate- and ALS-inhibiting herbicides, respectively (Castle et al., 2004; Green et al., 2008; Green, 2009). However, the dominant HR trait on the market is for glyphosate tolerance (Dill et al., 2008).

Glyphosate, an active ingredient in Roundup® agricultural herbicides, inhibits the biosynthesis of aromatic amino acids by suppressing the activity of the EPSPS enzyme, which is encoded by the *aroA* gene. The mutant allele of *aroA* located in *Salmonella typhimurium* resulted in the expression of a mutant EPSPS enzyme that was insensitive to glyphosate. The expression of this allele in transformed plants confers resistance to glyphosate in tobacco (Comai et al., 1985) and tomato (Fillatti et al., 1987), as well as induced the overproduction of EPSPS in petunia (Shan et al., 1986).

Abbreviations: ALS, acetolactate synthase; ANOVA, one-way analysis of variance; EFSA, European Food Safety Authority; EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase; FAO, Food and Agriculture Organization of the United Nations; GM, genetically modified; GOX, glyphosate oxidoreductase; ILSI, International Life Sciences Institute; HR, herbicide resistance; OECD, Organization for Economic Cooperation and Development; SD, standard deviation; SD rat, Sprague-Dawley rat; WHO, World Health Organization.

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Using these strategies, glyphosate resistance has also been achieved in soybean (Padgette et al., 1996), canola, cotton, and sugarbeet (Briggs and Koziel, 1998). Recently, a new mutant *aroA* gene encoding a highly glyphosate-resistant EPSPS was identified from *Pseudomonas fluorescens* strain G2, which was isolated from a storage area with a history of glyphosate pollution (Zhu et al., 2003). The *G2-aroA* gene conferred resistance to glyphosate when expressed in tobacco, corn, cole, and cotton (Dun et al., 2007). In China, the *G2-aroA* gene had been transformed into the maize genome, and the GM maize was demonstrated to be tolerant to glyphosate (data not published). In spite of the quick development of the technology throughout the world, China has taken the initiative in the research of transgenic plant, and this work will further promote those efforts.

Although GM technique has become popular over the last decade, there are still concerns about the safety of GM foods among the public. Several international organizations have developed a series of safety assessment processes to investigate the safety of foods or feeds obtained from genetically modified plants to humans and livestock (WHO, 1991, 1995; OECD, 1993, 1996, 1997; FAO, 1996; FAO/WHO, 2000; ILSI, 1996). The primary safety assessment of foods obtained from GM crops is based on the concept of substantial equivalence, whereby their composition is compared to that of their closest genetically related counterpart (i.e., near-isogenic control; OECD, 1993). In some cases, 90-day rodent feeding studies have been recommended to assess the potential for adverse effects of foods obtained from GM crops following long-term exposure (FAO/WHO, 2000; EFSA, 2008). In China, the 90-day feeding study in rodents is one of the primary requirements of the application for the safety certification of GM crops. A number of rodent feeding studies have been designed to determine whether the diets incorporated with grains from GM crops are substantially equivalent in composition and nutritional characteristics to non-transgenic control diets using standard toxicological response variables. The sensitivity of these studies to detect adverse effects has been documented (EFSA, 2008), and many GM events have undergone such studies, including tomato (Noteborn et al., 1995), rice (Wang et al., 2002; Schroder et al., 2007; Kroghsbo et al., 2008), soybean (Zhu et al., 2004; Appenzeller et al., 2008), cottonseed (Dryzga et al., 2007) and maize grain (Doull et al., 2007; Hammond et al., 2004, 2006a,b; Malley et al., 2007; Mackenzie et al., 2007; He et al., 2008, 2009; Appenzeller et al., 2009a,b). The results from these studies indicated that there were no adverse effects related to the insertion of one or more transgenic genes into the plant genome.

In the present study, the food safety of GM maize with the *G2-aroA* gene was assessed in a 90-day feeding study and compared with non-GM isogenic line. This study was conducted in compliance with the Chinese Toxicology Assessment Procedures and Methods for Food Safety (Chinese Standard GB15193.13-2003) and the OECD Good Laboratory Practice Guidelines at the Experimental Animal Center, Supervision and Testing Center for GMOs Food Safety, Ministry of Agriculture (Beijing, China). The experimental design was approved by the animal ethics committee of the center.

2. Materials and methods

2.1. Plant materials

The herbicide-resistant *G2-aroA* maize and its non-GM, near isogenic control were both cultivated in the experimental field in adjoining plots under identical climate conditions in the growing season of 2010 in Hainan Province of China.

2.2. Composition analysis and diet formulation of maize grain

The concentrations of the nutritional proximates (moisture, ash, fat, crude protein and fiber) in the GM maize and non-GM control were determined in accordance with standard methods (Chinese standard GB/T5009.3~6, 10-2003) and are shown in Table 1. Flours from GM and non-GM maize were formulated into rodent diets at concentrations of 12.5%, 25%, and 50% by Ke Ao Xie Li Feed Co. Ltd. (Beijing, China). An additional commercial diet was included as a negative control. The formulation of all diets is shown in Table 2. All diets were vacuum-packed and sterilized by ^{60}Co.

2.3. Animals

Seventy weaned male and seventy weaned female Sprague-Dawley rats were supplied by Vital River Laboratories Co. Ltd. (Beijing, China) with average body weights of 80–100 g. After acclimation for 5 days with reference diets, the rats were randomly divided into treatment groups with 10 rats/sex/group and mean body weights across each group varied within 20%. Every 5 rats were housed in a stainless steel cage with *ad libitum* access to water and feed. Animal room was maintained at a temperature of 22 ± 2 °C, relative humidity of 40% to 70%, artificially illuminated (fluorescent light) with a 12 h light/dark cycle and air exchanges of 15 times/h. The experiment was conducted in the Experimental Animal Center, Supervision and Testing Center for GMOs Food Safety, Ministry of Agriculture (Beijing).

2.4. Body weight gain and food utilization

The rats were observed daily for mortality and signs of toxicity or other notable phenomenon. Body weight and food intake were measured once a week.

2.5. Clinical evaluations

Variables of hematology and serum chemistry were measured in blood obtained from all rats on study day 90. Rats were fasted overnight (16 h), and blood samples were collected from the orbital sinus under anesthesia. The samples collected for hematology evaluation were placed in tubes containing EDTA·Na_2. The samples for serum chemistry evaluation were centrifuged at 4000*g* for 8 min, and the supernatants were collected individually.

2.6. Hematology

White blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell volume distribution (RDW), blood platelet count (PLT), and mean platelet volume (MPV) were measured with a HEMAVET 950FS animal blood cell counter (Drew Scientific, Inc., Dallas, Texas, USA).

2.7. Serum chemistry

Lactic Dehydrogenase (LDH), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), creatinine (CREA), blood urea nitrogen (BUN), glucose (GLU), calcium (Ca), phosphorus (P), total cholesterol (CHO) and triglycerides (TG) were measured with an automatic Biochemical Analyzer 7020 (HITACHI, Tokyo, Japan).

2.8. Pathology

At the end of the 13-weeks exposure test, a complete gross necropsy was performed on all animals by visual inspection. Some organs were selected for weighing, including brain, heart, lung, thymus, liver, spleen, kidney, adrenals, and testes or ovaries, with paired organs being weighed together.

Some selected tissues underwent histopathology examination, including brain, heart, lung, liver, adrenal glands, kidneys, stomach, small intestine (duodenum, jejunum, ileum), spleen, thyroids, ovaries and uterus or testes and epididymis, representing the major organs/systems. Paraffin-embedded tissues were sectioned to

Table 1
Nutritional components of conventional (non-GM) and transgenic maize flour (%, $n = 3$).

Nutrients	Non-GM maize	GM maize
Moisture[a]	3.61 ± 0.33	3.75 ± 0.18
Ash[b]	6.38 ± 0.21	6.74 ± 0.23
Protein[b]	20.67 ± 1.02	20.67 ± 0.12
Fiber[b]	3.18 ± 0.34	3.23 ± 0.40
Fat[b]	5.21 ± 0.51	5.84 ± 1.14

[a] Fresh weight.
[b] Dry weight.