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Safety evaluation of genetically modified DAS-40278-9 maize in a subchronic rodent feeding study

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ABSTRACT

Genetically modified (GM) maize, DAS-40278-9, expresses the aryloxyalkanoate dioxygenase-1 (AAD-1) protein, which confers tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) herbicides. The *aad-1* gene, which expresses the AAD-1 protein, was derived from Gram-negative soil bacterium, *Sphingobium herbicidovorans*. A 90-day sub-chronic toxicity study was conducted on rats as a component of the safety evaluation of DAS-40278-9 maize. Rats were given formulated diets containing maize grain from DAS-40278-9 or a non-GM near isogenic control comparator at an incorporation rate of 12.5%, 25%, or 50% (w/w), respectively for 90 days. In addition, another group of rats was fed a basic rodent diet. Animals were evaluated by cage-side and hand-held detailed clinical observations, ophthalmic examinations, body weights/body weight gains, feed consumption, hematology, serum chemistry, selected organ weights, and gross and histopathological examinations. Under the condition of this study, DAS-40278-9 maize did not cause any treatment-related effects in rats compared with rats fed diets containing non-GM maize.

1. Introduction

Maize (*Zea mays*) is a widely cultivated cereal that has been safely consumed by humans and animals for thousands of years (OECD, 2002). Competition from weeds can be a major source of reduced maize yield (Nader Soltani, 2016). Genetically modified (GM) crops offer farmers an alternative solution to improve crop tolerance to herbicides and thereby protect crop yield from loss due to weed competition. In the mid-1990's, herbicide tolerant (HT) crops were first developed using genetic modification techniques. HT crops have many benefits such as increased productivity, reduced pesticide use and greater flexibility in farm management and, as a result, have been broadly adopted. Broad adoption of HT crops and accompanying herbicide use has placed heavy selection pressure on weed species to also acquire herbicide tolerance. One mechanism for reducing selection pressure is use of herbicides with different modes of action (Vencill et al., 2012).

DAS-40278-9 maize is a HT trait developed by Dow AgroSciences to provide farmers an additional mode of action for weed management (Wright et al., 2010). DAS-40278-9 expresses the novel herbicide gene, *aad-1*, which was derived from a Gram-negative soil bacterium, *Sphingobium herbicidovorans*. The *aad-1* gene encodes the aryloxyalkanoate dioxygenase-1 (AAD-1) enzyme, conferring upon the plants tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (AOPP) herbicides (Wright et al., 2010). 2,4-D is a widely used broad spectrum herbicide. The aryloxyphenoxypropionate herbicides, also known as fop herbicides, constitute a family of grass-selective herbicides such as quizalofop. DAS-40278-9 maize is authorized for cultivation and food/feed use in United States, Canada and Brazil, and is also authorized for food and feed use in EU28, Australia, Colombia, Japan, Mexico, New Zealand, South Africa, South Korea, and Taiwan (ISAAA, 2016).

The safety of GM crops and their usage in food and feed is extensively and systematically evaluated prior to authorization for cultivation or food and/or feed use. One component of the safety assessment of individual GM crops in which a new protein is expressed is to evaluate the safety of the protein itself. This is accomplished by a comprehensive weight of evidence approach that includes history of safe use, assessment of the mode of action and intended effect of the protein, and resistance to degradation in the presence of digestive enzymes. In some cases, single and repeated dose animal toxicology studies have been conducted as well (CODEX, 2003; Delaney et al., 2008). All of these studies have been conducted with the AAD-1 protein and it

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showed no evidence of adverse effects (Stagg et al., 2012).

In addition to the safety assessment of the newly expressed proteins in the transgenic crops, a safety assessment of the whole food is also typically required. This assessment includes, a detailed compositional analysis to determine if the known constituents of a GM crop are present at concentrations that are present in conventional crop which is regarded as safe. A compositional analysis conducted with maize gran from DAS-40278-9 reported no consequential differences in comparison with its non-GM near isogenic comparator and numerous other commercially available non-GM reference lines (Herman et al., 2010).

In addition to the studies described above, feeding studies have been conducted with whole grains and processed food or feed fractions from GM crops in livestock and rodents to investigate whether unintended adverse effects could occur from consumption of them that may not have been anticipated from compositional testing (WHO, 1991; WHO, 1995). For maize grains from GM crops, many such feeding studies have been conducted in broiler chickens and rats and, to date, no evidence of adverse effects has been observed in either species (Herman et al., 2011; Li et al., 2015; Stagg et al., 2012; Zhu et al., 2013; Zou et al., 2016). No evidence of adverse nutritional effects was observed in broiler chickens fed maize grain from DAS-40278-9 (Herman et al., 2010). However, the results from rodent 90 day feeding studies, a standard rodent toxicology study have not yet been reported with the maize grain from DAS-40278-9.

In the current study, a subchronic (i.e., 90 day) feeding study was conducted with maize grain from DAS-40278-9 GM with Sprague Dawley (SD) rats by the Supervision, Inspection and Testing Center of Genetically Modified Organisms, Ministry of Agriculture (Beijing, China). The purpose was to evaluate the safety and nutritional equivalence of diets formulated with 12.5%, 25% or 50% DAS-40278-9 maize relative to diets containing the same concentration of grain from non-GM near isoline maize. The animal study was approved by the Animal Experimental Welfare and Ethical Inspection Committee (No. 130071–072) in the Supervision and Testing Center for GMO Food Safety at the Ministry of Agriculture (Beijing, China). Animals were cared for according to the Guide for the Care and Use of Laboratory Animals (Bayne, 1996), and the committees approved the protocols.

2. Materials and methods

2.1. Plant materials

The GM maize, DAS-40278-9, and non-GM maize grain were supplied by the Ministry of Agriculture (MOA) Science and Technology Development Center under the identifier JY130071-072. Both DAS-40278-9 and non-GM maize were in the 7SH3821*HHB genetic background. The grain from these two maize lines were from plants grown concurrently in the same location.

2.2. Diet formulation and experimental design

Each rat diet contained GM DAS-40278-9 or non-GM near isoline maize at the inclusion concentration of 12.5%, 25%, or 50% (w/w). The 50% inclusion rate is the highest incorporation rate for maize that would still allow proper nutritional balance (Fang et al., 2017; Han et al., 2016; Zhu et al., 2013). The diets were also fortified with other ingredients such as bean pulp, wheat flour, fishmeal, and yeast powder to ensure a balanced diet for the rats. The nutritional content of the diets were within the recommendations specified in the Chinese Standard GB14924.3–2010 (GB, 2010). All diets were vacuum-packed, irradiated with ^{60}Co by Keao Xieli Feed Co. Ltd (Beijing, China), and kept at 4–8 °C prior to use. Finally, the nutrient composition of diets was analyzed and evaluated (Table 1).

2.3. Animals and management

A total of 140 of SD rats weighing 80–100 g, at 4-weeks of age, were included in this study. All rats were purchased from Vital River Laboratory Animal Technology Co., Ltd (Beijing, China), and had a qualification certificate No. of SCXK, Beijing 2012–0001. After 5 days of acclimation, the rats were randomly divided into 7 groups based on body weight with 10 male and 10 female rats in each group. The rats were then fed with test diets for 90 days in the specific pathogen free (SPF) animal laboratory of the Supervision and Testing Center for Genetically Modified Organisms (GMOs) Safety, Ministry of Agriculture (SYXK, Beijing 2015–0046, China). The temperature and humidity of the animal room ranged from 20 to 24 °C and 40–70%, respectively. A 12 h light/dark cycle and air exchanges at 15 times/h were maintained to keep the environment suitable for the rats.

2.4. Groups

Six treatment groups were fed with diets containing 12.5%, 25%, and 50% (w/w) maize grain from DAS-40278-9 (T1, T2 and T3 groups) or non-GM near isoline maize (N1, N2 and N3 groups). The seventh group (CK group) was fed with a commercially produced standard laboratory animal diets made by Ke Ao Xie Li Feed Co., Ltd. (Beijing, China), with the license number SCXK (Beijing) 2014–0010. All ingredients were adjusted to meet the requirement of rats (GB 14924.3–2010) and the rodent feeds were exposed to ^{60}Co to keep them sterile. Water and diet were supplied *ad libitum*. Animal management and housing procedures were carried out in compliance with the OECD Principles of Good Laboratory Practice.

2.5. Clinical observation, body weight gain, and feed utilization

The rats were observed daily for clinical observations including their behavior, coat color, and other toxicity symptoms. At the beginning and at the end of the study, ophthalmic examinations were conducted on each animal. Feed consumption and body weight were recorded weekly. Feed utilization was determined using the following calculation:

Feed utilization (%) = (body weight gain/feed consumption) × 100%

2.6. Hematology

On day 91, the rats were fasted overnight and blood samples were collected from the orbital sinus under anesthesia using EDTA•K_2 as an anticoagulant. Subsequently, 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 using a HEMAVET 950FS (Drew Scientific, Inc., Dallas, TX, USA) automatic animal blood cell counter.

2.7. Serum chemistry

For serum collection, the blood samples were collected from the orbital sinus and centrifuged at *4000* × *g* for 15 min to separate the serum. Alanine aminotransferase (ALT), total protein (TP), albumin (ALB), alkaline phosphatase (ALP), glucose (GLU), blood urea nitrogen (BUN), creatinine (CREA), calcium (Ca), potassium (P), cholesterol (CHO), triglyceride (TG), lactate dehydrogenase (LDH), chlorine (Cl), and magnesium (Mg) were measured in the serum with an automatic Biochemical Analyzer 7020 (HITACHI, Tokyo, Japan).