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Subchronic feeding study of grain from herbicide-tolerant maize DP-Ø98140-6 in Sprague-Dawley rats

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ABSTRACT

This 13-week feeding study conducted in Sprague-Dawley rats evaluated the potential health effects from long-term consumption of a rodent diet formulated with grain from genetically modified (GM), herbicide-tolerant maize DP-Ø98140-6 (98140; trade name Optimum® GAT® (Optimum® GAT® is a registered trademark of Pioneer Hi-Bred)). Metabolic inactivation of the herbicidal active ingredient glyphosate was conferred by genomic integration and expression of a gene-shuffled acetylase coding sequence, *gat4621*, from *Bacillus licheniformis*; tolerance to acetolactate synthase (ALS) inhibiting herbicides was conferred by overexpression of a modified allele (*zm-hra*) of the endogenous maize ALS enzyme that is resilient to inactivation. Milled maize grain from untreated (98140) and herbicide-treated (98140+Gly/SU) plants, the conventional non-transgenic, near-isogenic control (091), and three commercial non-transgenic reference hybrids (33J56, 33P66, and 33R77) was substituted at concentrations of 35–38% w/w into a common rodent chow formula (PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002) and fed to rats (12/sex/group) for at least 91 consecutive days. Compared with rats fed diets containing grain from the conventional near-isogenic control maize, no adverse effects were observed in rats fed diets containing grain from 98140 or 98140+Gly/SU maize with respect to standard nutritional performance metrics and OECD 408-compliant toxicological response variables [OECD, 1998. Section 4 (Part 408), Health Effects: Repeated Dose 90-Day Oral Toxicity Study in Rodents, Guideline for the Testing of Chemicals. Organisation of Economic Co-operation and Development, Paris, France]. These results support the comparative safety and nutritional value of maize grain from genetically modified Optimum® GAT® and conventional, non-transgenic hybrid field corn.

1. Introduction

The current approach for the safety assessment of foods from new crop varieties developed using recombinant DNA technology relies on providing reasonable evidence that no harm will result from typical usage and consumption of foods from the new crop compared with similar foods from conventionally-bred crops (OECD, 1993). This concept of a *comparative* evaluation, known as the principle of substantial equivalence, was developed originally as part of the framework for safety assessment of the crops themselves (physiology, agronomics, composition), and has since become embedded into international regulatory guidelines for the safety assessment of *foods* and *feeds* from these crops (WHO, 1991, 1995; OECD, 1993, 1997, 2003; FAO, 1996; FAO/WHO, 2000; EC, 1997, 2003a,b, 2004; ILSI, 1997, 2003, 2004; Codex, 2003a, 2007; EFSA, 2006a,b). Implicit in this concept is that the assessment is of potential hazard, rather than a demonstration of absolute safety. In other words, foods and feeds from crops developed using recombinant DNA technology must be shown to be *as safe as* foods and feeds from conventional crops.

In practice, the process of evaluating substantial equivalence between a new genetically modified crop and its nearest isogenic relative is based on a comparison of molecular and morphological

Abbreviations: AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care; APHIS, Animal and Plant Health Inspection Service; DNA, deoxyribonucleic acid; EC, European Commission; EFSA, European Food Safety Authority; EPA, Environmental Protection Agency; FAO, Food and Agricultural Organisation; FDA, Food and Drug Administration; GLP, Good Laboratory Practices; Gly/SU, glyphosate plus nicosulfuron plus rimsulfuron; h, hour; ILAR, Institute of Laboratory Animal Resources; ILSI, International Life Sciences Institute; NAS, National Academy of Sciences; NRC, National Research Council; OECD, Organisation for Economic Cooperation and Development; PMI, Purina Mills International; ppm, part-per-million; SD, standard deviation; US, United States; USDA, United States Department of Agriculture; WHO, World Health Organisation; w/w, weight-to-weight.

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characteristics, agronomic performance metrics, and composition of nutritionally-important macro- and micronutrients, anti-nutrients, and other known bioactive metabolites characteristic of the food or feed product to detect possible coincident phenotypic effects that are not related to the intended alteration (Sidhu et al., 2000; OECD, 2002a,b; Ridley et al., 2002; ILSI, 2004; Herman et al., 2004, 2007; Obert et al., 2004; George et al., 2004; EFSA, 2006a; McCann et al., 2007; Drury et al., 2008; Lundry et al., 2008). When the genetic modification results in expression of heterologous proteins, the potential toxicity and allergenicity of these proteins are evaluated independently by considering results from *in silico*, *in vitro*, and *in vivo* toxicity studies (Harrison et al., 1996; Codex, 2003b; Herman et al., 2003; Hérouet et al., 2005; Delaney et al., 2008a,b).

Crop improvement using conventional or modern techniques, including recombinant DNA technology, could result in potentially adverse pleiotropic changes in nutritional value of the resulting food/feed products that may not be detected during compositional analysis (NAS, 2004). While fundamental safety standards should apply to all crop products entering the food supply, foods from crops developed using modern genetic modification techniques require a scientifically rigorous, systematic safety assessment to characterize potential health risks (EC, 1997; ILSI, 2004; Constable et al., 2007; Kok et al., 2008). Accordingly, the pre-commercialization safety assessment for new genetically modified crops often includes feeding trials in broiler chickens and other livestock species to address this possibility. The comparative nutritional wholesomeness of modified and conventional feeds is assessed using animal growth and carcass characteristics (Taylor et al., 2003a,b,c,d; Flachowsky et al., 2007; Huls et al., 2007; McNaughton et al., 2007a,b, 2008a,b; Jacobs et al., 2008). In some cases, 90-day (subchronic) repeated-dose toxicity studies in rats have been recommended as a more comprehensive bioassay to determine the health impact of aggregate changes to the food/feed matrix (OECD, 1997, 1998; Barlow et al., 2002; EFSA, 2006a,b). These studies evaluate standard toxicology response variables and to date have reported no biologically significant differences between animals fed food/feed products from genetically modified or near-isogenic conventional crop varieties (Hammond et al., 2004, 2006a,b; MacKenzie et al., 2007; Malley et al., 2007; Appenzeller et al., 2008; He et al., 2008; Healy et al., 2008).

Genetically modified Optimum® GAT® maize contains novel cisgenic and transgenic gene sequences in a molecular stack encoded by event DP-Ø9814Ø-6 (hereafter referred to as 98140). Endogenous acetyltransferase (AT) gene sequences were isolated from three related strains of the saprophytic soil bacterium *Bacillus licheniformis* for fragmentation-based multigene recombination ("gene shuffling") to create novel AT enzymes with high *in vitro* efficiency and specificity for the herbicidal active ingredient glyphosate (Castle et al., 2004; Siehl et al., 2005, 2007). On the same plasmid vector as the functionally-optimized *gat4621* (glyphosate AT) gene sequence, 98140 maize also contains the cisgenic sequence for a highly-resistant allele (*zm-hra*) of maize acetolactate synthase (ALS) that protects the branched-chain amino acid metabolic pathway from inhibition by sulfonylurea and imidazolinone herbicides (Challeff and Mauvais, 1984; Lee et al., 1988; Duggleby and Pang, 2000; Green, 2007). When integrated and expressed in 98140 maize, the GAT4621 and ZM-HRA proteins confer crop tolerance to both pre- and post-emergence weed management chemicals.

This paper presents the results from a rat subchronic dietary toxicity assessment of grain from Optimum® GAT® maize. Milled grain from 98140 and four conventionally-developed maize hybrids was incorporated separately into a common rodent diet formulation and fed to rats for at least 91 days. Growth performance and toxicological response variables were assessed comparatively in accordance with OECD 408 guidelines (OECD, 1998) to identify potential health hazards resulting from long-term dietary exposure to Optimum® GAT® maize grain.

2. Materials and methods

2.1. Test, control, and reference substances

Optimum® GAT® maize (98140), its non-transgenic near-isogenic control (091; a maize hybrid bred in parallel with 98140 but using non-transformed parental inbred lines containing neither *gat4621* nor *zm-hra*), and three non-transgenic commercially-available reference maize (field corn) hybrids (33J56, 33P66, and 33R77) were produced in separate plots within the same field in Richland, IA during the 2006 growing season. An additional plot of Optimum® GAT® maize (98140+Gly/SU) was treated with herbicides containing the active ingredients glyphosate (Touchdown HiTech®, Syngenta) and nicosulfuron plus rimsulfuron (Steadfast®, DuPont). Each grain lot was produced according to typical agricultural practices for commercial maize production, within compliance of applicable US guidelines for production of regulated crops (USDA-APHIS, 2008).

Milled grain samples from each of the six sources were subjected to quantitative real-time polymerase chain reaction (qRT-PCR) analysis utilizing a primer set specific for the DP-Ø9814Ø-6 event to confirm the molecular identity of the two test hybrids (Pioneer, Johnston, IA). Enzyme-linked immunosorbent assays (ELISAs) specific for each of the expressed transgenic proteins were performed to confirm expression of GAT4621 and ZM-HRA (Pioneer, Johnston, IA).

2.2. Compositional analysis

Control, reference, and test maize grain was analyzed for nutrient composition as well as anti-nutrient and contaminant levels to verify suitability for use in animal diets. EPL Bio-Analytical Services (EPL-BAS; Niantic, IL) conducted composition analysis under GLPs (nutrients: proximates, fiber [crude, neutral detergent, acid detergent], individual amino acids, minerals [Ca, P, Mg, K, Na, Zn, Mn, Cu, Fe], individual fatty acids, vitamins [B1/thiamine, B2/riboflavin, B6/pyridoxine, niacin, folic acid, vitamin E, and β-carotene], anti-nutrients [trypsin inhibitor activity, phytic acid], and secondary metabolites [coumaric acid, ferulic acid, inositol, raffinose, and furfural]). Additional vitamins (pantothenic acid) and minerals (Se) were quantified at Woodson–Tenent Laboratories (Woodson–Tenent; Memphis, TN). Mycotoxin concentrations (aflatoxins B1, B2, G1, and G2, zearalenone, moniliformin, cyclopiazonic acid, deoxynivalenol and its 3-acetyl and 15-acetyl derivatives, fumonisins B1, B2, and B3, and T-2 toxin) were determined at Romer Laboratories, Inc. (Romer; Union, MO), and oosporein and ergot metabolites (ergosine, ergotamine, ergocornine, ergocryptine, and ergocristine) were measured at University of Missouri Veterinary and Medical Diagnostic Laboratory (Columbia, MO). Columbia Food Laboratories (Corbett, OR) analyzed pesticide residues of chlorinated hydrocarbons (aldrin, BHC-alpha, BHC-beta, BHC-delta, chlordane, DDT-related substances, dieldrin, endrin, HCB, heptachlor, heptachlor epoxide, lindane, methoxychlor, mirex, and PCB) and organophosphates (diazinon, disulfoton, ethion, malathion, methyl parathion, parathion [ethyl], thimet, thiodan [endosulfan], and trithion).

2.3. Experimental diet formulation, composition, and characterization

Test, control, or reference grain was incorporated into rodent diets that were nutritionally and compositionally comparable to PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002. Purina TestDiet (Richmond, IN) produced six experimental diets incorporating 35–38% milled maize grain by weight from each hybrid (091, 098140, 098140+Gly/SU, 33J56, 33P66, or 33R77) in place of the commodity maize grain typically used.

All diets were analyzed quantitatively for nutrient composition and contaminants (summarized in Table 1). Nutritional proximates, fiber, amino acids, minerals (as above), and vitamins (as above, including E [as α-tocopherol], β-, γ-, δ-, and total tocopherols) were measured at EPL-BAS. Gross energy, additional vitamins (B12 [cobalamin], A [as retinol], D3 [cholecalciferol], choline, biotin, pantothenic acid), minerals (Se, Co, Cl, I, Cr, F), and heavy metals (As, Cd, Pb, Hg) were quantified at Woodson–Tenent. Mycotoxins and pesticide residues were measured as described above.

Samples from all diets were subjected to ELISA analysis for GAT4621 and ZM-HRA (Pioneer, Johnston, IA) to confirm identity of rodent diets containing milled maize grain from Optimum® GAT® corn and to verify absence of detectable transgenic contamination of the control and reference diets.

2.4. Animal care and management

The feeding trial was conducted at DuPont Haskell Global Centers for Health and Environmental Sciences (Newark, DE), an AAALAC-accredited facility, in accord with the Guide to the Care and Use of Laboratory Animals (ILAR,