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Spontaneous renal tumors in two rats from a thirteen week rodent feeding study with grain from molecular stacked trait lepidopteran and coleopteran resistant (DP-ØØ4114-3) maize

Jerry F. Hardisty [a,*], Deborah A. Banas [a], Chirukandath Gopinath [b], William C. Hall [c], Gordon C. Hard [d], Michihito Takahashi [e]

[a] Experimental Pathology Laboratories, Inc., Research Triangle Park, NC 27709, United States
[b] Consultant in Toxicology/Pathology, 53 High Street, Alconbury, Cambridgeshire PE28 4DP, United Kingdom
[c] Hall Consulting, Inc., 12337 Sherwood Forest Drive, Mount Airy, MD 21771, United States
[d] Private Consultant, 203 Paku Drive, Tairua 3508, New Zealand
[e] Pathology Peer Review Center, Hatanodai 6-23-2, Shinagawa-ku Tokyo 142-0064, Japan

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ABSTRACT

A thirteen week feeding study was conducted by feeding young adult male and female Sprague Dawley [Crl:CD®(SD)] rats diets containing grain from genetically modified (GM) DP-ØØ4114-3 maize that was either untreated (4114) or treated in the field with glufosinate ammonium (4114GLU). Control rats were fed diets containing the same concentration of near isogenic, non-GM maize grain (091) or one of three types of commercially available non-GM maize grain. At the end of the in-life phase, renal tubule tumors were reported in two male rats consuming diets containing 4114 maize grain. An expert panel of pathologists was convened as a Pathology Working Group (PWG) to review coded kidney histology sections from control (091) and treated (4114 and 4114GLU) male rats. The objectives were for the panel to characterize the histopathologic findings and to interpret their relationship to consumption of the indicated diet. The PWG concluded unanimously that the kidney tumors were characteristic of amphophilic-vacuolar (AV) tumors and AV atypical tubular hyperplasia which represent a distinctive phenotype that has been reported to occur sporadically in young Sprague Dawley Rats. The PWG determined that the neoplasms and atypical tubular hyperplasias were multicentric and bilateral which typifies tumors of familial origin. Degenerative/regenerative or cytotoxic changes consistent with nephrotoxicity leading to tumor induction were not observed in these rats and thus supports the conclusion that tumors were unrelated to consumption of the test diet. It was the unanimous opinion of the PWG that the proliferative renal tubule cell lesions were spontaneous and not related to consumption of diets containing 4114 maize grain.

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1. Introduction

Genetically modified (GM) maize containing event DP-ØØ4114-3 (4114) is resistant to certain Lepidopteran pests, including the European corn borer (*Ostrinia nubilalis*); resistant to certain coleopteran pests, including western corn rootworm (*Diabrotica virgifera virgifera*); and tolerant to glufosinate herbicide. These activities are attributable to a multigene DNA construct that drives expression of transgenic Cry1F, Cry34Ab1/Cry35Ab1, and PAT proteins, respectively. Previous studies have discussed the safety of these proteins (Herman et al., 2003; Hérouet et al., 2005; Ladics et al., 2006; Delaney et al., 2008; Juberg et al., 2009), the individual events in which the proteins were expressed (MacKenzie et al., 2007; Malley et al., 2007; He et al., 2008), and in a stacked trait product expressing all of these proteins that was produced with conventional breeding (Appenzeller et al., 2009).

A 13 week rodent feeding study was conducted to evaluate the potential health effects of exposure to rodent diets formulated with grain from 4114 maize in male and female Crl:CD®(SD) rats. Grain was generated for (1) an untransformed, near-isogenic control hybrid of the same breeding lineage that does not contain event DP-ØØ4114-3 (control substance 091); (2) test substance 4114 maize; (3) test substance 4114 maize treated in the field with glufosinate (4114GLU); and (4) 3 non-transgenic commercial maize hybrids (reference substances: 32D78, 33N29, and 34P88). All diets were formulated to contain 32% (wt/wt) of the respective maize grains at Purina TestDiet (Richmond, IN) in accordance with the standards of Purina Mills Labdiet® Certified Rodent LabDiet®

* Corresponding author. Tel.: +1 919 998 9407x600; fax: +1 919 998 9607.
E-mail address: jhardisty@epl-inc.com (J.F. Hardisty).

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Table 1
Study design of a 13 week rodent feeding study with grain from molecular stacked trait lepidopteran and coleopteran resistant (DP-ØØ4114-3) maize.

Group	Number/Group		Experimental diet descriptions
	Male	Female	
1	12	12	Non-transgenic near isogenic control (091)
2	12	12	Transgenic test (4114)
3	12	12	Transgenic test (4114GLU)
4	12	12	Non-transgenic commercial reference 1 (32D78)
5	12	12	Non-transgenic commercial reference 2 (33N29)
6	12	12	Non-transgenic commercial reference 3 (34P88)

5002 (PMI® 5002) and fed to rats for approximately 13 weeks in conformance with OECD Guidelines (No. 408; OECD 1998). A summary of the study design is presented in Table 1.

The results of the 13 week feeding study, demonstrating that no biologically significant test diet related adverse effects were observed, were reported in an accompanying publication (Delaney et al., 2012). During the study pathologist's examination of the protocol-required tissue sections renal tubule neoplasms were diagnosed in two male rats consuming diets containing the 4114 maize grain. Proliferative renal tubule changes were not observed in any of the other groups of male rats or in any of the female rats. To confirm this conclusion, the remainder of the renal tissue from all available male and female rats was step-sectioned and examined for tubular cell proliferative changes according to recommendations of the National Toxicology Program (Eustis et al., 1994). In accordance with the Environmental Protection Agency PR Notice 94-5, as a part of the Pathology Working Group (PWG) process, a pathology peer review, which included reexamination of all available sections of kidney from rats in all groups including the step sections was performed by a second pathologist who confirmed that there was no evidence of renal toxicity (Environmental Protection Agency, 1994).

The PWG was composed of the reviewing pathologist (Deborah A. Banas) and independent consulting pathologists (Gordon C. Hard, William C. Hall, Chirukandath Gopinath, and Michihito Takahashi) with expertise in the evaluation of rodent toxicity and carcinogenicity studies and with specific expertise in renal pathology. The purpose of the PWG was to characterize the proliferative renal tubule changes and to determine if they should be regarded as spontaneous or test diet related. This paper provides the results, discussion, and conclusions of the PWG review of the microscopic slides.

2. Methods

The PWG chairperson (Jerry F. Hardisty) organized and presented the material to the panel. The PWG Chairperson was a non-voting member during the PWG review and, in addition to organizing and presenting the material and slides to the PWG panel, was the author of the panel's report. The PWG examined all of the routine sections of kidney from all male rats in Groups 1, 2, and 3 (091, 4114, and 4114GLU, respectively). The slides were coded so that the treatment group and previous diagnoses were not known to the PWG. This allowed the PWG to independently diagnose the proliferative changes and to characterize the changes as hyperplasia, benign neoplasia, or malignant neoplasia, and to determine the nature of the changes without being influenced by the original diagnosis. Each participant recorded his/her diagnoses and comments on worksheets that were prepared by the Chairperson.

During the PWG review of the slides, the criteria used to diagnose rat kidney lesions were those recommended by the Society of Toxicologic Pathology (Hard et al., 1995). Diagnotic criteria for atypical tubule hyperplasia, an obligate precursor of renal tubule tumors, were those of Hard and Seely (2005). Nomenclature used for diagnosing rat kidney lesions have recently been prepared as part of a global initiative (INHAND – The International Harmonization of Nomenclature and Diagnostic criteria) supported by the Societies of Toxicologic Pathology in Europe, United States, and Asia (Japan, Korea, and India) (Frazier et al., 2012). These criteria were also considered and are available at the following website www.goreni.com.

After the slides had been examined, final PWG consensus diagnoses were recorded on the Chairperson's worksheets. The consensus diagnoses of the PWG were reached when the majority of the PWG participants were in agreement. Following the coded review of the routine section, step sections from any animal with proliferative lesions were examined by the PWG in an uncoded manner. The PWG was then asked to characterize the findings and interpret their relationship to feeding the test diet.

3. Results

During the coded review of the kidney sections from Groups 1, 2, and 3 male rats, the PWG confirmed the findings reported by the study and peer review pathologists that proliferative renal tubule lesions were present in only two Group 2 (4114) male rats. In the affected kidneys, the proliferative changes were multifocal and bilateral, and consisted of a spectrum of changes including both hyperplastic and neoplastic lesions. Atypical renal tubule cell hyperplasia and renal tubule cell adenomas were present in the routine sections of kidney. One of the two male rats had multiple renal tubule cell adenomas (Fig. 1) and several foci of atypical tubule cell hyperplasia (Fig. 2). The renal tubule cell adenomas were bilateral, all located in the outer cortex and consisted of small nodular masses. The adenomas were expansile, non-encapsulated masses organized into sharply-delineated lobules separated by a fine fibrovascular stroma. Occasionally central degeneration and necrosis was observed within individual lobules. The tumor cells were round to polyhedral with finely granular vacuolated eosinophilic to amphophilic cytoplasm. Lymphoid cell infiltrates were often present in the fibrovascular stroma within or adjacent to the proliferating cells. Foci of atypical tubular hyperplasia consisted of small circumscribed proliferations of similar cells that were limited to a single renal tubule. The second male rat had a single, very large nodular mass, diagnosed as tubule cell carcinoma involving the outer cortex and inner stripe of the outer medulla (Fig. 3), and two foci of atypical tubule cell hyperplasia in the outer cortex. The carcinoma was not as well circumscribed as the adenomas, but the neoplastic cells maintained a lobular pattern supported by a fine fibrovascular stroma. Adjacent to the lobular pattern of the AV tumor cells, the neoplastic cells were arranged in a tubular pattern with smaller more basophilic cells lining the tubules. Additional proliferative renal tubule lesions were present in the step sections taken from the residual wet tissue from these two animals. No proliferative renal tubule changes were present in any of the Group 1 (091), other Group 2 (4114), or Group 3 (4114GLU) male rats.

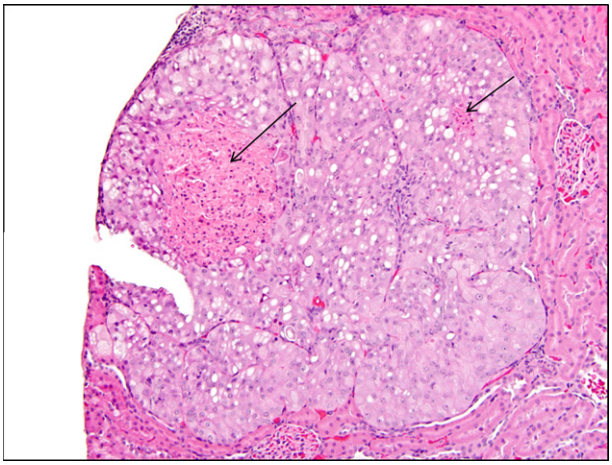

Fig. 1. Amphophilic vacuolated renal tubule adenoma containing lobules of eosinophilic to amphophilic vacuolated cells separated by a fine fibrovascular stroma. Focal necrosis is present in the center of two of the lobules (arrows).

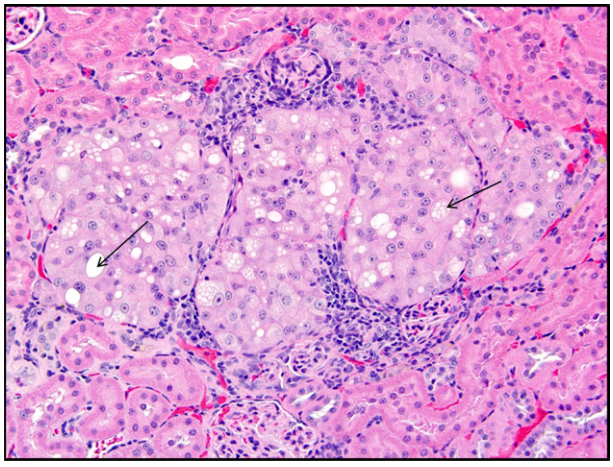

Fig. 2. Amphophilic vacuolated atypical tubule hyperplasia consisting of lobules of proliferating cells limited to a single renal tubule. The cells have amphophilic vacuolated cytoplasm (arrows) with a lymphocytic infiltration in the supporting stroma.

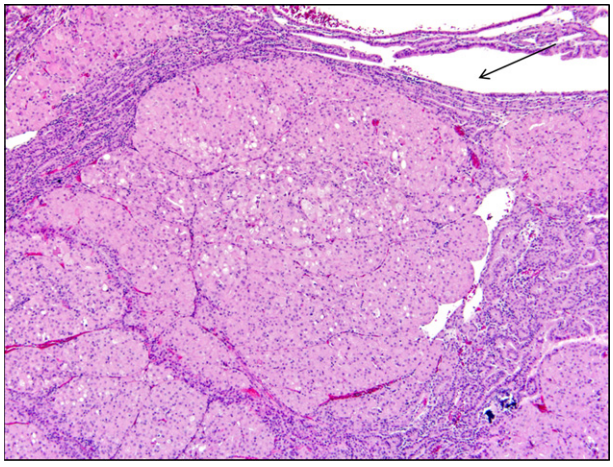

Fig. 3. Amphophilic vacuolated renal tubule carcinoma containing solid lobular proliferations of amphophilic vacuolated cells adjacent to smaller basophilic cells in a tubular pattern. Portions of the neoplasm are cystic (arrow).

Other than size, the proliferative lesions in both of these animals had similar morphologic characteristics. The proliferative lesions were located in the outer cortex extending in some instances to the inner stripe of the outer medulla. They were characterized as nodular masses composed of cords and nests of epithelial cells with solid and cystic growth patterns. The masses were generally non-encapsulated and divided into well-delineated lobules separated by a fine fibrovascular stroma containing lymphoid cells. The large mass in the second male rat had a few areas of central degeneration and necrosis. The tumor cells were large with large nuclei with prominent nucleoli and eosinophilic to amphophilic vacuolated cytoplasm. Occasional mitotic figures were present. At the margin of the large neoplasm, the neoplastic cells formed papillary cords which were slightly basophilic. Foci of atypical tubule hyperplasia (ATH) were also present in both kidney sections. The ATH were much smaller than the neoplasms and consisted of single tubules distended with multiple layers of proliferative lining epithelial cells which were similar in appearance to those present in the neoplasms, having enlarged nuclei, prominent nucleoli, and vacuolated eosinophilic to amphophilic cytoplasm.

4. Discussion

The proliferative kidney lesions reported in the slides from this 13 week rodent feeding study were limited to two male rats consuming diets containing the grain from 4114 maize. As reported by the study pathologist (Delaney et al., 2012), other than changes associated with chronic progressive nephropathy present in male rats in all three groups (091, 4114, and 4114GLU) with similar incidence and minimal severity, very few renal lesions were present. Male rats for Groups 4-6 and female rats examined by the study pathologist and peer review pathologist also had very few renal lesions except for nephropathy of generally minor severity (data not shown). There was no histological evidence of renal toxicity in the kidneys of any of the rats examined by the PWG or by the study and peer review pathologists.

The morphologic appearance of the foci of atypical renal tubule cell hyperplasia and renal tubule cell tumors described above in two male rats from the 4114 maize grain group were identical to that described for amphophilic, vacuolated types of proliferative renal tubule cell lesions (atypical tubule cell hyperplasia, renal tubule cell adenoma, and renal tubule cell carcinoma) that occur sporadically and have been determined to be spontaneous (Hard et al., 1994; Thurman et al., 1995; Hall et al., 2007; Lanzoni et al., 2007; Hard et al., 2008). These tumors have a distinctive morphologic presentation with hallmark features including eosinophilic/amphophilic staining cytoplasm, large finely granular cells, and numerous cytoplasmic vacuoles. They have been referred to as the amphophilic-vacuolar (AV) variant of renal tubule tumor. Renal tubule cell tumors with this distinctive histomorphology have been reported sporadically in several strains of young rats used in subchronic toxicity studies (thirteen-week or 90-day studies) (Hard et al., 1994), as well as in two-year carcinogenicity bioassays (Hard et al., 2008). They have been observed in control and treated groups, with no relationship to treatment, and have been considered spontaneously occurring tumors possibly related to genetic mutations with a familial predisposition (Thurman et al., 1995; ECETOX, 2002; Hall et al., 2007). Thus, it was the unanimous opinion of the PWG, that the proliferative renal tubule cell lesions that were present in the two 4114 males from this study were spontaneous and not related to the test diet.

In addition to the distinctive phenotype of the tumors present in the two 4114 males that is characteristic of amphophilic-vacuolar (AV) tumors, the lack of, degenerative, or cytotoxic changes is not consistent with nephrotoxicity. The absence of any nephrotoxicity which might lead to tumor induction supports the conclusion that these tumors were unrelated to the test diet. Renal tubule cell degeneration/regeneration or cytotoxicity consistent with nephrotoxicity was not observed. Additionally preneoplastic proliferative changes which would be expected with both genotoxic and non-genotoxic carcinogens were absent (Hard, 1998). Moreover, the AV neoplasms and AV atypical tubular hyperplasia present only in these two male rats were multicentric and bilateral, which can typify tumors of familial origin (Hard et al., 2008). None of these changes were seen in any other rats in the study. The lack of these changes is consistent with an underlying genetic susceptibility in these two affected rats that is completely unrelated to the treatment.

The unanimous conclusion of the PWG was that the proliferative renal tubule cell lesions were spontaneous and not related to consumption of diets containing grain from 4114 maize. The proliferative lesions in the kidneys of both of these male rats were characteristic of amphophilic-vacuolar (AV) tumors and atypical tubular hyperplasia, which represent a distinctive phenotype that is considered to have a familial predisposition. Additionally, degenerative/regenerative or cytotoxic changes consistent with nephrotoxicity leading to tumor induction were not identified in these

rats and therefore supports the conclusion that tumors were unrelated to treatment with the test diet. Moreover, the neoplasms and atypical tubular hyperplasia seen only in these two rats were multicentric and bilateral, which is characteristic of tumors of familial origin.

5. Conflict of Interest

The authors declare that have no conflict of interest.

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