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Renal histopathology in toxicity and carcinogenicity studies with *tert*-butyl alcohol administered in drinking water to F344 rats: A pathology working group review and re-evaluation

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ABSTRACT

An independent Pathology Working Group (PWG) re-evaluated the kidney changes in National Toxicology Program (NTP) toxicology/carcinogenicity studies of *tert*-butyl alcohol (TBA) in F344/N rats to determine possible mode(s) of action underlying renal tubule tumors in male rats at 2-years. In the 13-week study, the PWG confirmed that the normal pattern of round hyaline droplets in proximal convoluted tubules was replaced by angular droplet accumulation, and identified precursors of granular casts in the outer medulla, changes typical of alpha$_{2u}$-globulin (α_{2u}-g) nephropathy. In the 2-year study, the PWG confirmed the NTP observation of increased renal tubule tumors in treated male groups. Linear papillary mineralization, another hallmark of the α_{2u}-g pathway was present only in treated male rats. Chronic progressive nephropathy (CPN) was exacerbated in high-dose males and females, with a relationship between advanced grades of CPN and renal tumor occurrence. Hyperplasia of the papilla lining was a component of CPN in both sexes, but there was no pelvic urothelial hyperplasia. High-dose females showed no TBA-related nephrotoxicity. The PWG concluded that both α_{2u}-g nephropathy and exacerbated CPN modes of action were operative in TBA renal tumorigenicity in male rats, neither of which has relevance for human cancer risk.

1. Introduction

In the early 1990s, *tert*-butyl alcohol (TBA) was included in the toxicology studies of the National Toxicology Program (NTP), National Institutes of Health, because of its diversity of uses and various potential routes of human exposure (NTP, 1995). Primarily, TBA is an intermediate in the synthesis of some industrial chemicals, particularly in production of fuel oxygenates (McGregor, 2010). The main finding for the 13-week toxicity study was hyaline droplet accumulation. The results for male rats in the 2-year carcinogenicity bioassay as described in the NTP report included renal tumors of tubule origin, increased nephropathy, and for both males and females, transitional cell hyperplasia of the renal pelvic epithelium and presence of mineral deposits. The increase in renal tubule tumors in male rats was not statistically significant after analysis of results from single, standard sections of kidney, but was significant following step-sectioning of the kidney blocks (NTP, 1995). Given the uncertain state of knowledge at that time concerning modes of action underlying renal tumor development, the report on the TBA studies (NTP, 1995) was unable to draw definite conclusions regarding the occurrence of the renal tumors in animals exposed to TBA for 2 years. Based on the incidence of renal tumors, however, the NTP concluded that the study data reflected *some evidence of carcinogenicity* for TBA in the male rat (NTP, 1995). This was the only site for tumor formation in male rats, and there was no evidence of tumorigenesis in any organs of females (NTP, 1995).

The questions remaining centered around the following issues: whether the hyaline droplet accumulation at 13 weeks represented alpha$_{2u}$-globulin (α_{2u}-g) nephropathy; the presence or absence of granular casts (which normally accompany α_{2u}-g nephropathy at 13 weeks); the precise location of mineralized deposits at 2 years; the status of transitional cell hyperplasia of the renal pelvic epithelium as a possible separate manifestation of TBA toxicity;

Abbreviations: α_{2u}-g, alpha$_{2u}$-globulin; ATH, atypical tubule hyperplasia; A–V tumor, amphophilic–vacuolar tumor; CPN, chronic progressive nephropathy; H&E, hematoxylin and eosin; MH, Mallory Heidenhain; NTP, national toxicology program; PWG, pathology working group; TBA, *tert*-butyl alcohol.

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and linked to this latter point, the question of whether nephrotoxic effects of TBA were present in females as well as in male rats.

Since the 1995 NTP report, work on TBA in other laboratories has identified the accumulating protein as α_{2u}-g and observed an increase in tubule cell proliferation (Borghoff et al., 2001), as well as demonstrating reversible, non-covalent binding of TBA to α_{2u}-g (Williams and Borghoff, 2001). In addition, proliferative changes associated with the spontaneous kidney disease, chronic progressive nephropathy (CPN), have been investigated (Hard and Seely, 2005, 2006), and further diagnostic information on α_{2u}-g nephropathy (Hard, 2008) has become available. Accordingly, an independent Pathology Working Group (PWG) was organized to review the renal pathology in these toxicology studies to determine whether the questions concerning mode(s) of action responsible for renal tubule tumor development could be addressed in light of the accumulating information on hyaline droplet nephropathy and CPN.

2. Materials and methods

2.1. PWG location and participants

The PWG was conducted at the Archives of the NTP in Research Triangle Park, NC, during May 20–21, 2010. The PWG consisted of 5 voting members:

Dr. Richard H. Bruner, DVM, DACVP (Chairperson), Clemson, South Carolina.

Dr. Samuel M. Cohen, MD, PhD, DABP, FAToxSci, FIATP, Omaha, Nebraska.

Dr. Gordon C. Hard, BVSc, PhD, DSc, DACVP, FRCPath, FRCVS, FAToxSci, Tairua, New Zealand.

Dr. John M. Pletcher, DVM, DACVP, Potomac, Maryland.

Dr. Karen S. Regan, DVM, DACVP, DABT, Ashland, Ohio.

All participants were senior pathologists with extensive experience in chemically-induced nephrotoxicity and renal neoplasia in laboratory animals. Any use of the acronym PWG hereafter in this paper refers specifically to the deliberations of this independent group of pathologists.

2.2. Studies evaluated

The studies for which kidney sections were re-evaluated were the 13-week toxicity study of TBA (NTP Study No. 05142–01) and the 2-year carcinogenicity bioassay of TBA (NTP Study No. 05142–03), both in F344/N rats (NTP, 1995). In each study TBA had been administered in the drinking water and the doses were 0, 2.5, 5, 10, 20, and 40 mg/mL for the 13-week study for both males and females, and 0, 1.25, 2.5, and 5 mg/mL for males, and 0, 2.5, 5, and 10 mg/mL for females in the 2-year study. For the 13-week study, the PWG examined hematoxylin and eosin (H&E)-stained kidney sections from all animals in the 0 mg/mL (control) and 20 mg/mL groups (10 rats per group) as well as a representative sample of kidneys stained with Mallory Heidenhain (MH) stain from each of these groups. For the 2-year study, the PWG examined original (standard) H&E-stained kidney sections from all male rats in the control and high-dose groups (50 animals per group); all animals with renal tumors in all dose groups; a selection of animals with hyperplasia; and all control and high-dose female rats that had survived for longer than 700 days (29 control and 16 high-dose animals). In addition, all renal tubule tumors diagnosed by NTP in the step sections of kidney were examined.

2.3. Method of PWG evaluation

In all cases, the slides were evaluated without knowledge of group identity to preclude any possible bias. The diagnostic criteria used for rat renal lesions followed the guidelines published by the Society of Toxicologic Pathology (Hard et al., 1995, 1999) and the refinements concerning tubule proliferative lesions reported by Hard and Seely (2005, 2006) and Hard et al. (2008). The severity of CPN was graded on a scale of 1 to 4 (minimal, mild, moderate, and marked) based on percentage of kidney involved, similar to the convention used by the NTP.

In the 13-week study, the PWG assessed the presence of hyaline droplet accumulation in renal proximal tubules as well as the severity of CPN. The presence of exfoliated cells or tubular cell debris that would be indicative of early granular cast formation was also investigated. For the 2-year study, CPN severity was graded in all of the male rats in the control and high-dose groups as well as in the selection of female rats examined. In evaluating the control and high-dose animals, the PWG included assessment of the presence and distribution of renal mineralization and nature of any hyperplasia of the renal pelvis in both male and female rats, distinguishing between hyperplasia of the papilla lining, a manifestation of CPN, and hyperplasia of the renal pelvis urothelium.

Renal tumors were examined for morphological sub-type along with selected examples of renal tubule hyperplasia. Renal tubule tumors relevant to chemical-related induction are almost always basophilic in type. In contrast, renal tubule tumors/hyperplasias with an amphophilic–vacuolar morphology have been shown to be spontaneous in origin and not related to chemical exposure (Hard et al., 2008). Likewise, oncocytic tubule proliferations are considered to have no relevance to chemical exposure (Montgomery and Seely, 1990). For both the NTP and PWG results, the incidences of renal tubule tumors in the control and treated groups of male rats were compared statistically with the "Row by Column Contingency Table using the Chi-Squared Test of Independence (Ott, 2002).

With regard to tubule hyperplasia, members of the PWG differentiated between "simple" and "atypical" forms as recommended by the STP guidelines (Hard et al., 1995). In the studies under review, simple hyperplasia was regarded as a reactive tubular alteration directly associated with CPN. Atypical tubule hyperplasia was considered to represent a preneoplastic lesion with strong relevance to establishing the carcinogenic potential of the proliferative lesion. The PWG did not examine hyperplasias in step sections of the expanded study, and did not assign a severity grade to hyperplastic changes. Consequently, direct comparisons between NTP and PWG results for tubule hyperplasia were precluded by slight differences in diagnostic criteria and recording procedures.

2.4. PWG objectives

The specific objectives of the PWG were to:

1. Determine if additional histopathologic changes could be identified in the 13-week study that would assist in defining tumor pathogenesis;
2. Assess the biological significance of any kidney changes in high-dose female rats;
3. Determine the most appropriate diagnoses for the renal tumors and selected renal hyperplasias in the 2-year study, as well as assessing the impact of CPN on tumor occurrence;
4. Provide additional comment on the mode(s) of action underlying the male rat kidney tumors; and
5. Provide perspective concerning human risk assessment based upon the PWG findings.

3. Results

3.1. 13-Week study – male rats (NTP Study No. 05142-01)

All PWG pathologists agreed that hyaline droplet accumulation was increased with increasing dose-levels of TBA in the cytoplasm