UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

November 28, 2018

To: John Bucher, NTP

From: Kristina Thayer, NCEA-IRIS

Subject: Pathology consult for ETBE and tBA

Purpose

The purpose of this memo is to request a consult for pathology-related issues discussed in the ethyl tertiary butyl ether (ETBE) and tert-butyl alcohol (tBA) draft IRIS assessments. This request is being conducted under the existing MOU between EPA NCEA and the National Toxicology Program (NTP) that covers cooperation and communication in the development of human health toxicological assessments.

Background

The draft IRIS assessments identify kidney effects as a potential human hazard of ETBE and its metabolite tBA, primarily based on evidence in rats (ETBE and tBA Sections 1.2.1, 1.3.1). EPA evaluated the evidence, including the role of α2u – globulin (in accordance with EPA guidance [U.S. EPA, 1991]) and chronic progressive nephropathy (CPN; for which no formal guidance is available). tBA was determined to induce α2u -globulin mediated nephrotoxicity, however, for ETBE, although increased hyaline droplets of α2u -globulin were observed, data were insufficient to conclude that ETBE induces α2u-globulin nephropathy (only one of the five steps in the pathological sequence, linear mineralization, was consistently observed). Both chemicals show dose-related exacerbation of CPN (increased incidence and/or severity), as well as lesions that are not specifically defined as CPN (increased urothelial hyperplasia of the renal pelvis and suppurative inflammation) but are reported to be associated with late stages of CPN (Frazier et al., 2012). Thus, EPA selected urothelial hyperplasia/transitional epithelial hyperplasia of the renal pelvis as the basis for the reference values for both ETBE and tBA.

The SAB committee reviewing ETBE and tBA was unable to reach a consensus with respect to how the EPA interpreted the ETBE and tBA databases for noncancer kidney effects. There was disagreement within the SAB as to whether any noncancer kidney effects for ETBE and tBA should be considered a hazard relevant to humans. Specifically, the difference in opinion was related to the extent of confidence in the roles that CPN and/or α2u-globulin-based mechanisms played in the development of the renal effects seen with tBA and ETBE.

Charge Questions

In this pathology consult, IRIS is seeking additional input on the role that α2u-globulin and CPN play in the observed kidney toxicity. Please consider the following questions and provide references, as applicable, with your responses. Please also comment on any sex-related aspects that are pertinent to these questions.

- Is the etiology of CPN in rats known?
- Are urothelial hyperplasia of the renal pelvis and transitional epithelial hyperplasia of the renal pelvis considered to be the same lesion?
- Suppurative inflammation and urothelial hyperplasia have been reported to be associated with advanced stages of CPN (Frazier et al 2012). Does NTP agree with this conclusion? Are these lesions also associated with α2u -globulin nephropathy?
- CPN exacerbation has been reported in some chemicals that NTP identified as candidates for acting via the α2u-globulin pathway (Travlos et al., 2011). A theory has been proposed that CPN exacerbation seen in male animals with ETBE and tBA exposure is caused by α2u-globulin related processes. Please comment on the strength of the above proposition.
- It has been hypothesized that there is no analog to the CPN process in the aging human kidney. Does this position reflect the consensus in the field of pathology?
- Given what is known about the biology of CPN development in rodents, is it plausible a chemical which exacerbates CPN in rats could also exacerbate disease processes in the human kidney (e.g. diabetic nephropathy, glomerulonephritis, interstitial nephritis)?

References

Frazier KS, Seely JC, Hard GC, Betton G, Burnett R, Nakatsuji S, Nishikawa A, Durchfeld-Meyer B, Bube A. 2012 Proliferative and nonproliferative lesions of the rat and mouse urinary system. Toxicol Pathol. 40(4 Suppl):14S-86S.

Travlos GS, Hard GC, Betz LJ, Kissling GE. 2011. Chronic progressive nephropathy in male F344 rats in 90-day toxicity studies: its occurrence and association with renal tubule tumors in subsequent 2-year bioassays. Toxicol Pathol. 39(2):381-9.

U.S. EPA 1991. Alpha-2u-globulin: Association with chemically induced renal toxicity and neoplasia in the male rat. EPA/625/391/019F.

Attachments

JPEC (Japan Petroleum Energy Center). (2010a). Carcinogenicity test of 2-Ethoxy-2-methylpropane in rats (Drinking water study). (Study No: 0691).

JPEC (Japan Petroleum Energy Center). (2010b). Carcinogenicity test of 2-Ethoxy-2-methylpropane in rats (Inhalation study). (Study No: 0686).

Kristina Thayer

_____

Kristina Thayer, Ph.D.
Director, NCEA-IRIS

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709
Website: http://www.niehs.nih.gov

February 13, 2019

Kristina Thayer, Ph.D.
Director, NCEA-IRIS
U.S. Environmental Protection Agency
109 T.W. Alexander Drive, MD B243-01
Research Triangle Park, NC 27709

Dear Dr. Thayer,

With respect to your November 28, 2018 request for a pathology consult under the NTP/NCEA Memorandum of Understanding, I asked Dr. Robert Sills, Chief, Cellular and Molecular Pathology Branch to provide responses reflecting the current NTP perspective on the issues you raise. Dr. Sills worked with John Curtis Seely, DVM Diplomate, ACVP Senior Pathologist Experimental Pathology Laboratories, Inc, an internationally recognized expert in rodent renal pathology, to provide answers to your questions.

1. Is the etiology of CPN known?

The etiology of CPN is unknown (Peter et al., 1986; Hard and Khan, 2004; Hard et al., 2013). Although several theories have been postulated to be the etiology of CPN none have been recognized as the absolute cause of CPN. Factors which have been suggested to be associated with the etiology of CPN include genetics, increased glomerular permeability and dysfunction due to hyperfiltration and functional overload, high renal protein levels, and hemodynamic changes. All of these may influence the progression of CPN but do not appear to initiate renal CPN disease (Baylis, 1984; Barthold, 1998; Abrass, 2000; Hard and Khan, 2004). CPN is a spontaneous and complex degenerative/regenerative disease process influenced by age (incidence and severity increases with age), sex (males affected more than females), and strain (in order of highest to lowest CPN incidence: Sprague-Dawley →Fischer 344 →Wistar rats). It can be modified by diet (increased protein and high caloric intake), hormones (testosterone, estrogen), and many other factors (Seely et al., 2018).

2. Are urothelial hyperplasia of the renal pelvis and transitional epithelial hyperplasia of the renal pelvis considered to be the same lesion?

Yes, the older terminology of “transitional epithelium hyperplasia, renal pelvis” is being updated and replaced by the newer terminology of “urothelial hyperplasia, renal pelvis”. Urothelium is recognized as the correct terminology of the epithelium lining the renal pelvis, ureter, urinary bladder and a portion of the urethra (Frazier and Seely, 2018). However, in advanced stages of CPN a type of epithelial proliferation/hyperplasia may be observed along the epithelial lining of the

renal papilla which in some older studies was designated as “urothelial hyperplasia”. Recently, the epithelial lining of the renal papilla has been unequivocally demonstrated to represent a type of epithelium different from the urothelium lining the renal pelvis. The difference between urothelium (uroplakin positive) and the epithelium lining the renal papilla (uroplakin negative) was confirmed by immunostaining for uroplakin (a distinct cell marker for urothelium) (Souza et al., 2018).

3. Suppurative inflammation and urothelial hyperplasia have been reported to be associated with advanced stages of CPN (Frazier et al., 2012). Does NTP agree with this conclusion? Are these lesions also associated with α2u-globulin nephropathy?

   Renal inflammation is not uncommon in the laboratory rat and can be observed throughout all portions of the kidney. Within the pelvis, inflammation tends to result in a reactive hyperplasia of the urothelium (Seely et at., 2018). Most cases of suppurative inflammation and urothelial hyperplasia are observed as spontaneous changes of undetermined origin. Interstitial mononuclear cell infiltrates are commonly observed in advanced stages of CPN (Frazier and Seely, 2018). However, suppurative inflammation and urothelial hyperplasia are typically unrelated to CPN or, at most, occasionally noted as an uncommon secondary change to CPN. Therefore, CPN does not directly result in suppurative inflammation or urothelial hyperplasia of the renal pelvis in its advanced stages. Cases of suppurative inflammation and urothelial hyperplasia are more likely to be associated with the presence of renal pelvic mineralization, pelvic calculi, or from an ascending bacterial infection or pyelonephritis (Seely et al., 2018). Furthermore, mineralization has been reported to be associated with an increased incidence and severity of spontaneous inflammation and urothelial hyperplasia in the renal pelvis of female rats (Tomonari et al., 2016). In addition, there is no information that appears to support that suppurative inflammation and pelvic urothelial hyperplasia are directly associated with the spectrum of morphological changes associated with α2u-globulin nephropathy (Frazier et al., 2012; Frazier and Seely, 2018).

4. CPN exacerbation has been reported in some chemicals that NTP identified as candidates for acting via the α2u-globulin pathway (Travlos et al., 2011). A theory has been proposed that CPN exacerbation seen in male animals with ETBE and tBA exposure is caused by α2-globulin related processes. Please comment on the strength of the above proposition

   According to the IARC Scientific Publication No. 147 (1999), chemicals which cause α2u-globulin nephropathy are often associated with an accelerated onset and severity (exacerbation) of the cortical changes typical of chronic progressive nephropathy seen in older male rats (Alden et al., 1984; Swenberg and Lehman-McKeenan, 1999; Travlos et al., 2011; Frazier et al., 2012). However, studies on 2-ethoxy-2 methylpropene (ethyl tertiary butyl ether; inhalation and drinking water studies) confirmed the presence of exacerbated CPN in both male and female rats at the highest dose levels (Japan Industrial Safety and Health Association/Japan Bioassay Research Center, 2010[a] 2010[b]). Because of “urothelial hyperplasia” and linear pelvic (papillary) mineralization noted in the male rats from these studies, it was proposed that α2u-globulin nephropathy contributed to the exacerbation of CPN in the males although no pathogenesis of the exacerbated CPN in females was given. Additionally, in these studies, “urothelial hyperplasia” was apparently and according to its description more likely to represent a proliferation of the papillary lining epithelium and not representative of true “urothelial hyperplasia”. This proliferative epithelial finding is often observed

as part of advanced cases of rat CPN and has no similarity to any human renal papillary finding (Seely et al., 2018; Souza et al., 2018). Long term exposures to methyl tertiary -butyl ether also resulted in an α2u-globulin nephropathy and exacerbated CPN in both male and female rats (Cruzan et al., 2007). The etiology of exacerbated CPN in females is not known since α2u-globulin nephropathy is regarded as a male only condition. Therefore, although α2u-globulin nephropathy may account for cases of chemically exacerbated CPN, other undetermined factors contributing to CPN exacerbation cannot be discounted (Doi et al., 2007).

5. It has been hypothesized that there is no analog to the CPN process in the aging human kidney. Does this position reflect the consensus in the field of pathology.

   Yes, the publication by Hard, Johnson, and Cohen makes a very strong case that the renal development, biological behavior, and morphological spectrum of CPN have no analog in the human kidney and that CPN is a distinct entity in the rat. (Hard et al., 2009). Overall, CPN has prominent protein filled dilated tubules, no vascular changes, no immunological or autoimmune basis, and little inflammation which distinguishes CPN from most human nephropathies (Hard et al., 2009). There appears to be nothing in the literature that counters this assumption.

6. Given what is known about the biology of CPN development in rodents, is it plausible a chemical which exacerbates CPN in rats could also exacerbate disease processes in the human kidney (e.g. diabetic nephropathy, glomerulonephritis, interstitial nephritis)?

   The etiology of CPN is unknown and represents a complex disease process in rats. Given the fact that there is no definitive pathogenesis for this multifactorial disease process, it cannot be fully ruled out that chemicals which exacerbate CPN in rats may have the potential to exacerbate disease processes in the human kidney.

   Please let me know if you have additional questions or wish further clarification of any of these responses.

   Sincerely,

   John Bucher

John Bucher, Ph.D.
National Toxicology Program,
NIEHS

References:

Abrass CK. 2000. The nature of chronic progressive nephropathy in aging rats. Adv Renal Replacement Therapy 7(1): 4-10.

Alden CL, Kanerva RL, Ridder G, Stone LC. 1984. The pathogenesis of the nephrotoxicity of volatile hydrocarbons in the male. In: Renal Effects of Petroleum Hydrocarbons, Advances in Modern Environmental Toxicology, Vol 7. MA Mehlman, CP Hemstreet, JJ Thorpe, NK Weaver (eds). Princeton Sci Pub, Princeton, NJ, pp. 107-120.

Barthold SW. 1998. Chronic progressive nephropathy, rat. In: Urinary System. 2nd ed. TC Jones, GC Hard, U Mohr (eds), pp.228-233, Springer-Verlag, Berlin.

Baylis C. 1994. Age-dependent glomerular damage in the rat: dissociation between glomerular injury and both glomerular hypertension and hypertrophy. Male gender as a primary risk factor. J Clin Invest 94:1823-1829.

Capen CC, et al. (eds) 1999. Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis. IARC Scientific Publications No. 147, International Agency for Research on Cancer, Lyon, France.

Cruzan G, Borghoff SJ, de Peyster A, Hard GC, McClain M, McGregor DB,Thomas MG. 2007. Reg Toxicol Pharmacol 47: 156-165.

Doi AM, Hill G, Seely J, Hailey JR, Kissling G, Bucher JR. 2007. $\alpha 2_{u-}$ globulin nephropathy and renal tumors in National Toxicology Program studies. Toxicol Pathol 35:533-540.

Frazier KS, Seely JC, Hard GC, Betton G, Burnet R, Nakatsuji S, Nishikawa A, Durchfeld-Meyer B, Bube A. 2012. Proliferative and nonproliferative lesions of the rat and mouse urinary system. Toxicol Pathol 40 (4 Suppl): 14S-86S.

Frazier KS, Seely JC. 2018. Urinary system. In: Toxicologic Pathology: Nonclinical Safety Assessment. 2nd ed. PS Sahota, JA Popp, PR Bouchard, JF Hardisty, C Gopinath (eds), CRC press/Taylor and Francis, Boca Raton, pp. 569-638.

Hard GC, Khan KN. 2004. A contemporary overview of chronic progressive nephropathy in the laboratory rat and its significance for human risk assessment. Toxicol Pathol 32:171-180.

Hard GC, Johnson KJ, Cohen SM. 2009. A comparison of rat chronic progressive nephropathy with human renal disease-implications for human risk assessment. Crit Rev Toxicol. 39(4):332-346.

Japan Industrial Safety and Health Association/ Japan Bioassay Research Center. 2010[a]. Carcinogenicity Test of 2-ethoxy-2-methypropene in Rat (Drinking Water Study (Study 0691).

Japan Industrial Safety and Health Association/Japan Bioassay Research Center. 2010[b]. Carcinogenicity Study of 2-ethyl-2-methylpropene in F344 Rats (Study 0686).

Peter CP, Burek JD, van Zwieten MJ. 1986. Spontaneous nephropathies in rats. Toxicol Pathol 14(1):91-100.

Seely JC, Hard GC, Blankenship B. 2018. Kidney. In: Boorman's Pathology of the Rat. AW Suttie, JR Leininger, AE Bradley (eds), pp. 125-166, Elsevier, Academic Press.

Souza N, Hard G, Arnold L, Foster K, Pennington K, Cohen S. 2018. Epithelium lining rat renal papilla: nomenclature and association with chronic progressive nephropathy (CPN). Toxicol Pathol 46(3):266-272.

Swenberg JA, Lehman-McKeenan LD. 1998. α_{2-} Urinary globulin-associated nephropathy as a mechanism of renal tubule cell carcinogenesis in male rats. 1998. In: Species Differences in Thyroid, Kidney, and Urinary Bladder Carcinogenesis. CC Capen, E Dybing E, JM Rice, JD Eilbourn (eds). IARC Scientific Publications No. 147. IARC, Lyon pp. 95-118.

Tomonari Y, Kurotaki T, Sato J, Doi T, Kokoshima H, Kanno T, Tsuchitani M, Seely JC. 2016. Spontaneous age-related lesions of the kidney fornices in Sprague-Dawley rats. Toxicol Pathol 44: 226-232.

Travlos GS, Hard GC, Betz LJ, Kissling GE. 2011. Chronic progressive nephropathy in male F344 rats in 90-day toxicity studies: its occurrence and association with renal tubule tumors in subsequent 2-year bioassays. Toxicol Pathol 39:381-389.