

## Response to Comments from Dr. Sam Cohen Regarding Chronic Progressive Nephropathy (CPN) in Rats and Its Relevance to Humans:

I feel compelled to respond to Dr. Cohen's remarks regarding the relevance of kidney injury data in rats from exposures to ETBE and tBA. The nature of his remarks are not very respectful of the job with which the EPA and its scientists are charged in protecting human health. I certainly do not disparage the need for pathology expertise, the implication in Dr. Cohen's comments is that without such expertise, the CAAC's recommendations are without merit. This is simply untrue.

My points are listed below. Some key statements are highlighted.

1. One does not have to be a pathologist to make an appropriate judgement as to whether there is sufficient data to rule out the relevance of kidney effects in rats from ETBE and tBA exposure to human risk assessment. When one considers a database such as that for ETBE and tBA that is not particularly robust, caution should be exercised and I believe one should err on the side of protecting human health. Thus, I believe that insufficient evidence exists to conclude with any degree of reasonable certainty that kidney responses in rats exposed to ETBE or tBA are irrelevant to humans.

2. *Review from Melnick et al. (2012):*

[Melnick, R.L., Burns, K.M., Ward, J.M., and Huff, J. (2012) Chemically Exacerbated Chronic Progressive Nephropathy Not Associated with Renal Tubular Tumor Induction in Rats: An Evaluation Based on 60 Carcinogenicity Studies by the National Toxicology Program. *Toxicol. Sci.* **128**, 346-356.]

*Overall Conclusion:*

"The large number of chemicals that induce severe CPN but do not induce renal tubular tumors (RTTs) in rats points to a major deficiency in the attempts to causally link advanced CPN to RTT development: inconsistency in the proposed association. Thus, empirical evidence provides no support for the claim that exacerbated CPN is predictive of RTT induction in rats. Because kidney cancer rates in humans are increasing and the causes of this disease are not fully known, it is essential, from a public health perspective, to regard environmental and occupational exposures to agents inducing kidney cancers in experimental animals as posing a carcinogenic risk to humans."

*Some Key Points:*

- If severe CPN causes renal tumorigenesis, it would be expected that RTTs would be commonly observed in control animals fed the high-protein NIH-07 diet. However, the rates of RTTs were low (about 1% or less) in control male F344 rats fed NIH-07 in the 2-year studies; and less than 0.5% in control female F344 rats.

- For at least nine chemicals that had some or clear evidence for carcinogenic activity in the kidney, the increased incidences of RTTs in the low- or mid-dose groups occurred with no or very slight increases in mean CPN severity compared with their control groups or with mean CPN grades indicative of only mild to moderate severity. The lack of comparable dose–response relationships contradicts the hypothesis that exacerbated CPN is causative of neoplastic effects in the kidney.
- CPN is not an established mode of action or mechanism of renal carcinogenicity. Neither the etiology of this kidney disease in aging control rats nor the mechanism of chemically exacerbated CPN in rats is known.
- Based on an evaluation of the potential influence of toxicity on the outcome of NTP’s carcinogenicity studies, histopathological findings alone cannot justify or confirm mechanistic assumptions of chemically induced tumors.
- Studies of some chemicals demonstrated a clear lack of association between exposure-related increases in severity of CPN and RTTs in male rats.
- Chemically exacerbated CPN in rats is very often not associated with increased incidences of RTTs.
- In the case of t-butyl alcohol, moderate to marked CPN was observed with increased incidences of RTTs in male rats (though with apparently different dose–response relationships), whereas exacerbated CPN in female rats was not associated with induction of RTTs.
- Chronic renal fibrosis, a lesion observed in rat CPN, is seen in human kidneys, with uncertain associations with renal cancer.

3. *Study by Okuda et al. (1986) showing features of Adriamycin-induced nephropathy in rats as a model for chronic progressive glomerular disease seen in humans:*

[Okuda, S., Oh, Y., Tsuruda, H., Onoyama, K., Fujimi, S., and Fujishima, M. (1986) Adriamycin-induced nephropathy as a model of chronic progressive glomerular disease. *Kidney Int.* **29**, 502-510.]

*Abstract:*

Serial changes in urine protein, blood chemistry, and histology of the kidney were investigated in rats for 28 weeks after injections of adriamycin (ADR). Massive proteinuria, hypoalbuminemia, and hyperlipidemia were observed at week 4 and throughout the experiment. Both BUN and serum creatinine began to increase at week 16 and reached the uremic level at week 28. Light microscopic study of the kidney demonstrated a normal appearance at week 4, vacuole formation in glomerular tuft at weeks 8 and 12, focal and segmental glomerular sclerosis at weeks 16 and 20, and extensive glomerular sclerosis with tubulointerstitial degenerations at weeks 24 and 28. Immunohistologically, IgM with a small amount of IgG and C3 appeared in the sclerosing glomeruli from week 16. Aggregated human IgG, injected intravenously at week 24, had accumulated mainly in the glomeruli. Electron microscopy revealed degenerative changes of glomerular epithelial cells with small vacuoles in the cytoplasm at week 4. Size of vacuoles increased at the later stage. In conclusion, ADR produced chronic, progressive glomerular changes in rats, which led to terminal renal failure.

The segmental glomerular sclerosis and IgM dominant glomerular deposition in these animals are similar to pathological characteristics of focal and segmental glomerular sclerosis seen clinically.

4. JPC Systemic Pathology Urinary System (Dec 2017)

[https://www.askjpc.org/vspo/show\\_page.php?id=655](https://www.askjpc.org/vspo/show_page.php?id=655)

[from the Joint Pathology Center, Silver Spring, MD: The JPC provides cost-effective, centralized, pathology subspecialty expertise; the sole veterinary pathology training source for the US military; Stewardship of a tissue repository unparalleled in size, age, and diversity. We serve the Department of Defense, the Department of Veterans Affairs, and other federal agencies. We partner with US and international academic and scientific institutions to advance pathology research and education.]

The entire website content is shown below; note the highlighted text regarding comparative pathology (towards end of material).

**Signalment (JPC #1565530): Rat**

**HISTORY:** Tissue from an old laboratory rat

**HISTOPATHOLOGIC DESCRIPTION:** Kidney: Diffusely, all levels of the nephron are moderately to severely affected by the following changes. There is a mild reduction in the number of glomeruli and multifocally those remaining have one or more of the following changes: The basement membrane of Bowman's capsule is variably thickened and hyalinized and there is periglomerular fibrosis; the parietal epithelium of Bowman's capsule is hypertrophied; there are multifocal adhesions of the glomerular tuft to Bowman's capsule (synechia); the uriniferous space is dilated; there is segmental or global glomerulosclerosis with multifocal obsolescence of glomerular tufts. Diffusely within the cortex and medulla, tubules have one or more of the following changes: swollen epithelium with indistinct cell borders and microvacuolated eosinophilic cytoplasm (degeneration); marked tubular ectasia with attenuation and/or loss of epithelium; abundant basophilic cytoplasm with vesiculate nuclei (regeneration) that occasionally piles up and forms irregular tubules (hyperplasia), with multifocally thickened basement membranes; or shrunken atrophic tubules with attenuated epithelium. Ectatic tubule lumina often contain one or more of the following: variable amounts of pale granular to brightly eosinophilic homogenous proteinaceous material (proteinosis, hyaline casts), deeply basophilic granular material (mineralization), sloughed epithelial cells with cellular and karyorrhectic debris (necrosis), few degenerate neutrophils, and rare erythrocytes. Tubular epithelia, primarily of the proximal convoluted tubule, multifocally contain variably distinct, irregularly round, intracytoplasmic 1-4 um diameter eosinophilic hyaline droplets. Diffusely, the interstitium is moderately expanded by fibrosis, edema, and multifocal infiltrates of low numbers lymphocytes and plasma cells. Multifocally there is a small amount of light golden-brown material (hemosiderin) within the interstitium and tubules. Multifocally, the capsular surface is irregular and undulant.

**MORPHOLOGIC DIAGNOSIS:** Kidney: Glomerulosclerosis, segmental to global, multifocal, marked, with synechia, periglomerular fibrosis, tubular degeneration, necrosis, and regeneration, marked tubular ectasia with proteinosis, and chronic interstitial nephritis, rat, rodent.

**CONDITION:** Chronic progressive nephropathy (CPN)

**SYNONYMS:** Glomerulosclerosis, progressive glomerulonephrosis, “old rat nephropathy”, protein overload nephropathy, chronic renal disease, chronic nephritis, dietary nephritis, glomerulonephritis, chronic progressive glomerulonephropathy, glomerular hyalinosis, progressive renal disease, spontaneous nephrosis, others

**GENERAL DISCUSSION:**

- **The most common, life-limiting disease of aged rats, and the most important renal disease in rats and mice;** prevalence may exceed 75% in susceptible strains
- Progressive disease that can lead to chronic renal failure and death
- Predisposing factors:
  - **Age:** usually seen in rats **>12 months old**
  - **Sex:** more prevalent in **males**
  - **Strain:** higher prevalence in Sprague-Dawley and Fischer 344 rats
  - **Diet: high protein diets** predispose to disease; total dietary caloric restriction (rather than dietary protein restriction alone) delays onset and reduces disease progression
  - Immunologic factors: affected glomeruli have mesangial deposition of IgM consistent with noncomplement-fixing immune complexes; however, CPN does not appear to be primarily an immune-mediated disease
  - **Endocrine:** prolactin levels implicated as a contributing factor
  - **Microbial status:** axenic rats tend not to develop CPN and therefore live much longer than microbe-associated rats
- Generally considered a **degenerative** to atrophic disease with compensatory **regenerative** hyperplasia
- Lesions can be evident as early as 3 months, but do not usually become severe until over 52 weeks of age; age of onset, incidence, and severity varies with stock/strain
- Lesions are typically bilaterally symmetric
- CPN contributes to hypertension and is often associated with polyarteritis nodosa
- Rats cope well with disease, but may rapidly decompensate and die

**PATHOGENESIS:**

- Pathogenesis is complex and exact mechanism is unknown; essentially any nephrotoxic insult can result in CPN; it is suggested that glomeruli are the initial site of injury
- Fibrosis:
  - **Macrophages and myofibroblasts** appear to play an important role in the development of interstitial fibrosis, likely through production of TGF- $\beta$
  - TGF- $\beta$  plays an important role in the development of fibrosis, which can be minimized with sirtuin therapy (AJP 2017)
- In advanced cases, secondary hyperparathyroidism often develops, resulting in metastatic mineralization in multiple organs (e.g. kidney, gastric mucosa, lungs, and media of larger arteries) and osteodystrophy with fibroplasia

- Increased glomerular protein loss à functional overload of nephrons à “hyaline” droplets in tubular epithelial cells with associated increased lysosomal activity

#### **TYPICAL CLINICAL FINDINGS:**

- Weight loss, proteinuria
- Advanced cases: elevated plasma creatinine (renal insufficiency)
- **Nephrotic syndrome** in advanced cases: marked proteinuria, hypoalbuminemia, edema, hypercholesterolemia
  - Elevated serum cholesterol and marked proteinuria (>300mg/dL) are useful diagnostic parameters

#### **TYPICAL GROSS FINDINGS:**

- Renal cortices pitted, sometimes irregular, with irregularities and linear streaks in the cortex and medulla on cut surface with varying degrees of brown pigmentation
- Kidneys variably enlarged, pale

#### **TYPICAL LIGHT MICROSCOPIC FINDINGS:**

- Changes consistent with chronic glomerulonephropathy are present at all levels of the nephron; lesions depend on chronicity
  - Early lesions: focal to multifocal foci of tubule basophilia, nuclear crowding, peritubular basement membrane thickening, variable infiltration by mononuclear inflammatory cells
  - With progression: Amount of affected renal parenchyma increases, individual components of CPN become more severe, and hyaline casts are prominent, as well as development of glomerular changes such as capillary tuft thickening, adhesions between glomerular epithelium and Bowman’s capsule (synechiae), and glomerulosclerosis; there may be a small but significant increase in proliferative lesions of the proximal tubule
- **Tubules:**
  - Intraluminal **proteinaceous or hyaline casts** in dilated tubules of the cortex and medulla
  - Eosinophilic PAS-positive and iron-positive resorption droplets (“hyaline” droplets) frequently present in tubular epithelium of affected nephrons
  - Tubules often either ectatic and lined by attenuated epithelium, or contracted and lined by poorly differentiated cuboidal **basophilic epithelium**, or sclerotic
  - Variable thickening and splitting of proximal tubular basement membrane
- **Glomeruli:**
  - Lesions vary from minimal basement membrane thickening to marked thickening of glomerular tufts with segmental **glomerulosclerosis** and adhesions to Bowman’s capsule (synechiae)
  - Variable thickening and splitting of Bowman’s capsular basement membrane
- **Interstitial:**
  - Interstitial fibrosis

- Mononuclear cell infiltration
- Renal papilla: Epithelial hyperplasia with advanced disease
- **Other Tissues:** Metastatic mineralization of the kidney, gastric mucosa, lungs, and media of larger arteries in severe cases due to renal secondary hyperparathyroidism

#### ULTRASTRUCTURAL FINDINGS:

- Thickened capillary basement membranes
- Distorted, fused, and enlarged podocyte foot processes distort distortion, enlargement, and fusion of podocyte foot processes in multiple areas

#### DIFFERENTIAL DIAGNOSIS:

##### Histologic:

- Chronic bacterial pyelonephritis: medullary necrosis, patchy fibrosis in outer medulla and cortex, inflammatory exudate within renal pelvis
- Renal ischemic injury: necrosis (acute) or fibrosis (chronic) of entire nephrons
- Nephrosis associated with toxic insults (e.g. aminoglycosides): cortical tubules uniformly affected
- **Atypical tubule hyperplasia** and adenoma: CPN can have tubular proliferative lesions; atypical hyperplasia or adenoma are expansive with complex proliferation of tubules and no thickened basement membrane

#### COMPARATIVE PATHOLOGY:

- **Mice, hamsters, gerbils, guinea pigs:** Age-related chronic renal disease morphologically similar to CPN of rats has been diagnosed in a variety of laboratory rodents; in rats, basement membrane thickening occurs in the absence of vascular lesions, which is unlike these rodents; mice have less severe renal and secondary systemic changes (e.g., metastatic calcification)
- **Humans: Diabetic glomerulopathy and end-stage renal disease**
- **Common marmosets:** Spontaneous progressive glomerulonephropathy dominated by glomerular lesions with secondary tubulointerstitial lesions is similar to CPN in rodents; pathogenesis unknown
- **Dogs: X-linked hereditary nephropathy** of Navasota dogs due to a genetic defect resulting in **defective production of type IV collagen**, resulting in similar disease with mesangioproliferative glomerulopathy and progressive proteinuria with glomerular loss, interstitial fibrosis, tubular necrosis, and variable inflammation
- **Naked mole rat:** Similar progressive chronic nephritis/nephropathy is common in aging animals of this long-lived species; glomerular changes are less severe than in rats and mice with CPN and characterized predominantly by membranous glomerulopathy, Bowman capsule dilation is not a feature; secondary hyperparathyroidism is not a feature of this disease
- **Key Largo woodrat:** similar disease

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**5. Final Comments:**

My position is that the database for ETBE and tBA regarding kidney effects is insufficient to rule out potential relevance for humans. Hence, the human kidney should be considered a relevant target organ for ETBE and tBA.