EPA's Response to Interagency Comments on the Final Interagency Science Discussion Draft of the IRIS Toxicological Review of *tert*-Butyl Alcohol (*tert*-Butanol)

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Purpose: The Integrated Risk Information System (IRIS) assessment development process of May 2009 includes two steps (Step 3 and 6b) where the Executive Office of the President and other federal agencies can comment on draft assessments. Comments on the Final Interagency Science Discussion draft of the IRIS Toxicological Review of *tert*-Butyl Alcohol (*tert*-Butanol) were provided by the National Institute for Occupational Safety and Health (NIOSH) and the Office of Management and Budget (OMB), none of which NIOSH and OMB considered to be major scientific comments. The following are EPA's responses to the primary interagency comments. All interagency comments were taken into consideration in revising the draft assessment prior to posting on the IRIS database.

For a complete description of the IRIS process, including Interagency Science Discussion, visit the IRIS website at www.epa.gov/iris.

Interagency Science Discussion Comments and Responses:

Alpha2u-globulin/CPN clarifications – NIOSH commented that the role of alpha2u-globulin in renal toxicity and carcinogenesis should be clearly stated. NIOSH pointed to text in the assessment under *Overall Conclusions on Mode of Action for Kidney Effects* that they read to suggest that the relevance of renal tumors for humans remains unclear because the evidence is inconclusive on whether the nephropathy and carcinogenicity are due to alpha2u-globulin or other processes.

> **EPA Response:** EPA agrees that the relative contribution of alpha2u-globulin processes to tert-butanol renal toxicity and carcinogenesis is unclear. Due to this uncertainty, EPA has focused on noncancer kidney effects in female rats (to avoid confounding by alpha2u-globulin processes). In the cancer section EPA acknowledges that alpha2u-globulin plays some role in kidney tumors, but cancer risk estimates are not derived for kidney tumors in male rats. This conclusion is based on the following (U.S. EPA, 1991a): "if some renal tumors in male rats are attributable to the alpha 2u-globulin process and some are attributable to other carcinogenic processes, such tumors remain relevant for purposes of hazard identification, but a dose-response estimate based on such tumors in male rats should not be performed unless enough information is available to determine the relative contribution of each process to the overall renal tumor response."

EPA has revised text in the *Overall Conclusions on Mode of Action for Kidney Effects* section to clarify the current understanding and remaining uncertainty regarding the role of alpha2u-globulin in kidney toxicity and carcinogenesis caused by *tert*-butanol exposure.

NIOSH and OMB suggested clarifying statements around chronic progressive nephropathy (CPN) and human relevance in the kidney section. NIOSH commented that the rationale for why kidney lesions in male rats were not considered for human hazard identification is not clear.

EPA response: EPA has revised text in the assessment to improve the clarity around alpha2u-globulin and CPN, as well as the relationship between the two. As suggested by OMB, EPA adapted the following text from the ETBE assessment:

"While the etiology of CPN is unknown (Hard, 2004; NIEHS, 2019; Peter, 1986) and it has no known analog in the aging human kidney (Hard, 2009; NIEHS, 2019), it cannot be ruled out that a chemical which exacerbates CPN in rats could also exacerbate disease processes in the human kidney (e.g. chronic kidney disease, diabetic nephropathy, glomerulonephritis, interstitial nephritis, etc) (NIEHS, 2019)."

"In addition, no known counterpart to CPN has been identified in the aging human kidney. However, several individual lesions noted in CPN (e.g. tubule atrophy, tubule dilation, thickening of tubular basement membranes, glomerulosclerosis) also occur in the human kidney (Frazier, 2012; Lusco, 2016; Zoja, 2015; Satirapoj, 2012; NIEHS, 2019). Therefore, exacerbation of one or more of these lesions following ETBE exposure may reflect some type of cell injury or inflammatory process, which is relevant to the human kidney."

EPA also revised text on CPN to clearly summarize NTP's conclusions: "Given the fact that there is no definitive pathogenesis for CPN, 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 (NIEHS, 2019)."

OMB commented that EPA has not made the case that male kidney tumors are attributable to other carcinogenic process besides alpha2u-globulin.

EPA Response: Oral exposure to *tert*-butanol in rats resulted in an increase in kidney tumors in males, but no kidney tumors were found in females. As discussed in Section 1.2.1, some of these tumors might be associated with alpha2u-globulin nephropathy. Evidence in support of this hypothesized MOA includes the accumulation of hyaline droplets in renal tubule cells, the presence of alpha 2u-globulin in the hyaline droplets, and additional aspects associated with alpha 2u-globulin nephropathy, including linear papillary mineralization and foci of tubular hyperplasia. Other evidence, however, is not supportive: the accumulation of hyaline droplets was minimal, concentrations of alpha 2u-globulin were low at doses that induced tumors, and no significant necrosis or cytotoxicity was observed

in association with compensatory regenerative proliferation or induction of granular casts within a time frame consistent with alpha 2u-globulin-mediated nephropathy

EPA also evaluated CPN as a hypothesized MOA using EPA's Cancer Guidelines (U.S. EPA, 2005). This analysis is found on pages 1-48 to 1-51. EPA found that CPN and the exacerbation of CPN play a role in renal tubule nephropathy. The available evidence indicates that CPN is involved in the induction of kidney tumors in male rats, likely by providing proliferative stimulus in the form of compensatory regeneration following toxicity to the renal tubule epithelium, although these effects were not observed in some studies.

Kidney tumors are associated with CPN, but the data on CPN are not coherent; dose-response relationships for CPN, renal tubule hyperplasia, and renal tubule tumors differed. In addition, CPN was nearly as severe in female rats as in male rats, yet no female rats developed renal tumors. Thus, some renal tumors might be attributable to alpha 2u-globulin nephropathy augmented by CPN, and some to other, yet unspecified, processes. Taken together, and according to EPA's guidance on alpha2u-globulin (U.S. EPA, 1991a), kidney tumors in males induced by *tert*-butanol are relevant for human hazard identification. The SAB did not reach consensus on EPA's conclusion. The SAB recommended strengthening text around the justification for EPA's conclusion, which was done using expert consultation provided by NTP (NIEHS, 2019).

Clarity on justification on not deriving an IUR- NIOSH commented that the rationale for not deriving an IUR should be clearly stated. NIOH stated that Section 2.4 does not provide a conclusion on the feasibility of deriving and IUR

EPA response: EPA agrees that the rationale for not deriving an IUR was unclear in the draft. Although an OSF was derived based on suggestive evidence in rats and mice, no quantitative cancer estimate of risk was derived for inhalation exposures. Route to route extrapolation (oral to inhalation) was not possible because a PBPK model for mice was not available for *tert*-butanol. Furthermore, U.S. EPA's guidance on alpha2u-globulin (U.S. EPA, 1991a) states that if some renal tumors are attributable to alpha 2u-globulin while others may be due to other processes then these tumors remain relevant for hazard identification, but that a quantitative estimate should not be performed unless there is sufficient evidence to determine the relevant contribution of each processes to the development of tumors . EPA has revised the text in Section 2.4 to clarify why an IUR was not derived.

Kidney endpoint selection for reference values. OMB commented that the rationale for the selection of different kidney endpoints between ETBE and *tert*-butanol is not clear.

EPA response: EPA agrees that the justification of kidney endpoint selection was unclear in the draft. Although *tert*-butanol and its parent compound ETBE both have effects on the kidney, there are specific differences in the databases that limit the selection of endpoints. For ETBE, consistent changes in kidney weight and urothelial hyperplasia were observed across multiple studies and kidney weight was considered a better representation of the database although urothelial hyperplasia was a more sensitive endpoint. t*ert*-Butanol has a more limited database and three POD's were considered: increased severity of nephropathy, suppurative inflammation, and absolute kidney weight. Increased severity of nephropathy was chosen over other potential endpoints because it is considered a more specific marker of impaired kidney function and the PODs were all within 2-fold of each other. In most cases EPA prefers to use a BMDL; however, that does not preclude other database considerations. While the use of increased severity of nephropathy utilizes a LOAEL which adds uncertainty, an $UF_L = 3$ was used for this endpoint since the magnitude of change at the LOAEL was determined to not require a full 10-fold extrapolation to approximate a NOAEL or BMDL. EPA has revised the text in Section 2.1.4 to clarify the endpoint selection for deriving reference values.