National Institute for Occupational Safety and Health (NIOSH)
Comments on the Interagency Science Discussion Draft
IRIS Assessment of *tert*-Butyl Alcohol July 2020
(Date Received August 19, 2020)

[Comments received via email. Substantiative comments summarized in EPA's Response to Selected Interagency Comments on the EPA IRIS Website]

Comments from the National Institute for Occupational Safety and Health (NIOSH) on the Environmental Protection Agency (EPA) Toxicological Review of *tert*-Butyl Alcohol (*tert*-Butanol) dated July 2020, EPA/635/R-20/105a, Final Agency and Interagency Draft (144 pages)

August 19, 2020

General comments:

NIOSH finds the EPA's current risk decisions for oral and inhalation non-cancer effects well-reasoned and reflective of the evidence. The logic behind the selection of the Lowest Observed Adverse Effect Level (LOAEL) for increased nephropathy in female rats as a point of departure is clearly stated. Given the endpoints examined, toxicokinetic considerations, and respective study durations, the EPA's use of the chronic oral study in female F344 rats as a basis for an inhalation reference concentration (RfC) is well-supported. NIOSH notes that the EPA's comprehensive documentation of the toxicokinetic transformations used to derive the inhalation dose threshold would be useful for others conducting risk assessments for *tert*-butanol in the future.

For the determination of oral and inhalation cancer risks, the body of data is limited in terms of the number of individual studies, total species examined, the toxicokinetic information available for those species that are tested, and the modes of action (MOAs) of observed treatment-related tumors. EPA summarizes these limitations and their impacts, and given this body of data NIOSH finds EPA's decision to use thyroid tumors in female mice as the basis for an oral cancer slope factor supported by the evidence, with the caveat that it is generally not ideal to base this value on an effect that is observed in only one species and the underlying MOA is not known. NIOSH has questions regarding the derivation of the inhalation unit risk for cancer in the line comments below.

Specific comments/questions:

xvi, line 71: Change "facilitates" to "facilitate."

xxiv, line 14: The abbreviation "POD" was not defined earlier in the document; it should be spelled out and defined at first use.

1-15, lines 2-4: "$B6C3F_1$ mice, however, did not exhibit histopathological changes when exposed for 13 weeks and 2 years via the oral route (NTP, 1995) and 13 weeks via the inhalation route (NTP, 1997)." The rest of this section discusses the results of rat studies in detail; however, the lack of effect in mice is not discussed.

1-18, lines 37-38: "This effect in females, which was not considered toxicologically significant, is not discussed further." NIOSH suggests adding the criteria used to decide that the effect was not considered toxicologically significant, so readers know how this decision was reached.

1-19, lines 9-12: "The group's report and analysis by Hard et al. (2011) confirmed the NTP findings of renal tubule hyperplasia and renal tubule tumors in male rats at 2 years. In particular, they reported similar overall tumor incidences in the exposed groups. Hard et al. (2011), however, reported fewer renal tubule adenomas and carcinomas in the control group than in the original NTP study." The report from Hard et al. (2011) re-evaluates the NTP 1995 study and confirms the alpha2u-globulin-induced nephropathy. However, the report highlights that the transitional hyperplasia observed may not be a nephrotoxic response. Also, suppurative inflammation, based on their histopathological evaluation, was reported as an effect of bacterial infection rather than *tert*-butanol-induced toxicity.

1-37, lines 19-22: "These results from NTP (1997), which are inconsistent with the findings of both Borghoff et al. (2001) and NTP (1995), do not appear to be due to differences in dose." The inconsistencies may be attributed to the difference in route of exposure. Although it is explained in lines 21-25 on page 1-37 that the average blood concentration is comparable in both the studies, it is possible that route of exposure influences the mechanism of action and this point needs to be discussed when explaining the inconsistencies.

1-37, lines 25-27: "The absence of similar histopathological findings in the 13-week inhalation NTP (1997) study compared to those reported in the two oral studies is not understood, but might be indicative of the strength of *tert*-butanol to induce, consistently, alpha2u-globulin nephropathy." The difference between oral and inhalation study outcomes may be due to differences in route of exposure. This is particularly true since blood concentrations of *tert*-butanol were similar between oral and pulmonary exposures. This topic should be discussed in the document.

1-38, lines 2-17: Regarding the information reported from Borghoff et al. (2001), NIOSH suggests the dose(s) used (i.e., the statistically significant increases of a2-microglobulin by ELISA) be stated in this summary so that the reader may more easily understand how the data do or do not demonstrate dose-response concordance for a2-microglobulin accumulation being a key event. Regarding the statement that ELISA is a more sensitive method of detection for macroglobulin increases than IHC: please clarify whether this is EPA's conclusion or that of Borghoff et al. (2001), and how this is known.

1-43: There are several uses of *t*-butanol instead of *tert*-butanol. Usually, acronyms are used consistently throughout a document.

1-44, lines 12-15: "(b) Biochemical information regarding binding of the chemical to the alpha2u-globulin protein: Williams and Borghoff (2001) report that tert-butanol reversibly and noncovalently binds to alpha2u-globulin in the kidneys of male rats. This provides additional support to the involvement of the alpha2u-globulin process.". The finding that *tert*-butanol reversibly and noncovalently binds to alpha2u-globulin in the kidneys provides additional support to the involvement of alpha2u-globulin is not a convincing argument. First, it is not stated if the binding is specific or non-specific. If it is not specific, that weakens the conclusion. Second, has the binding of *tert*-butanol to alpha2u-globulin been established to be necessary for pathological outcome?

1-44, lines 32-35: "The few studies available to assess the direct genotoxic potential of tert-butanol primarily are negative, although a few studies report DNA damage induced by oxidative stress. DNA damage induced by oxidative stress is consistent with the decreased levels of glutathione in male rat kidneys reported by Acharya et al. (1995) after 10 weeks of tert-butanol exposure." Sgambato et al. (2009), mentioned on Page 1-42, lines 23-25, have shown that *tert*-butanol can induce DNA damage, nuclear fragmentation and effects on cell cycle and expression of cyclins and p53 in rat fibroblasts. Although DNA damage effect seemed to disappear after 4 hours, the differential expression of cell cycle proteins and tumor suppressor genes is indicative of *tert*-butanol's possible genotoxic effect. These findings suggest that *tert*-butanol is capable of inducing carcinogenic events; however, the MOA is unclear. As mentioned above, oxidative stress-induced DNA damage is one of many possible MOAs for carcinogenesis. It is important to include all possible evidence for *tert*-butanol-induced toxicity before evaluating its carcinogenic potential.

1-45, lines 35-37: "Although the evidence suggests that *tert*-butanol induces alpha2u-globulin nephropathy, the data indicate that *tert*-butanol is a weak inducer of alpha2u-globulin and that this process is not solely responsible for the renal tubule nephropathy and carcinogenicity observed in male rats." Based on the statement above, the evidence is inconclusive on whether the nephropathy and carcinogenicity are due to the alpha2u-globulin MOA or to toxicity of *tert*-butanol itself. Therefore, the relevance of the renal tumors for humans remains unclear.

1-78, lines 4-10: This paragraph is confusing as currently written. In the "Mode of Action Analysis—Kidney Effects" section, EPA determines that the a2-microglobulin accumulation pathway is a minor contributor to kidney effects (renal tubule nephropathy and tumors). In the present section integrating the body of data, EPA states that "Because alpha2u-globulin nephropathy contributes to CPN, CPN and CPN-associated lesions in male rats were not considered for human hazard identification." If the rat-specific microglobulin MOA were determined to be an obligatory for these lesions rather than a minor contributing event, it would follow that these lesions are not relevant to human health. Because it is only a minor contributing event, the language in the above statement is not logical. Furthermore, the observance of chronic progressive nephropathy (CPN) and other kidney effects in female rats (discussed in the next paragraph beginning with line 11) demonstrates that these effects are caused by events not dependent on a2-microglobulin, but rather a "spectrum of toxicities" that may be relevant to human kidney. Lesions in male rats are therefore relevant to identifying hazards to human health even though the data may present limitations as far as estimating a dose-response threshold for human risk.

2-26, lines 4-18: EPA notes here that there is insufficient toxicokinetic knowledge to extrapolate *tert*-butanol exposure from the oral to inhalation route in the mouse, so the mouse study used for the oral slope factor cannot be used to derive an inhalation unit risk value. However, this paragraph also states that an inhalation unit risk (IUR) value based on the NTP (1995) study in F344 rats using the established PBPK model was not attempted because the relative contributions of the male rat-specific a2-microglobulin accumulation MOA could not be defined. NIOSH has the following questions:

1) Earlier in the assessment, the rat PBPK model is used to derive an internal dose from oral exposure and EPA deemed it appropriate to limit the dataset to female rats in order to estimate a point of departure for noncancer kidney effects. Why is that approach not sufficient here?
2) Section 2.4 does not seem to offer a conclusion on the feasibility of deriving an IUR value at this time. Given the limitations of the dataset and the available tools for analyzing dose response, does EPA conclude that a value is not possible at this time? Please clarify EPA's position on these aspects in the text.

R-5, lines 17-19: the link goes to a 1978 Occupational Health Guideline, not to a 1992 document.

A-1, table A-1 in the Supplemental Information document (129 pages): Please change "National Institute of Occupational Safety and Health" to "National Institute **for** Occupational Safety and Health." In the same row, in the "Toxicity value" column, NIOSH suggests including the NIOSH short-term exposure limit and the Immediately Dangerous to Life and Health (IDLH) value provided in the NIOSH Pocket Guide to Chemical Hazards: https://www.cdc.gov/niosh/npg/npgd0078.html.