MIBK and tert-butyl ethanol (TBA) have been grouped for discussion because of their similar toxicology. Both show evidence of inducing α2u-globulin-nephropathy, an accumulation of protein in rat kidney tubules that leads to inflammation and tumors through a non-genotoxic mechanism. This condition only occurs in male rats and so its relevance for hazard assessment is questioned. Seven criteria have been established to confirm that male rat kidney tumors are a result of α2u-globulin-nephropathy:

- Lack of genotoxic activity (agent and/or metabolites) based on an overall evaluation of in vitro and in vivo data,
- Male rat specificity for nephropathy and renal tumorigenicity,
- Induction of the characteristic sequence of histopathological changes in shorter- term studies, of which protein droplet accumulation is obligatory,
- Identification of the protein accumulating in the tubule cells as α2u,
- Reversible binding of the chemical or metabolite to α2un,
- Induction of sustained increased cell proliferation in the renal cortex, and
- Similarities in dose-response relationship of the tumor outcome with the histopathological end-points (protein droplets, α2u accumulation, cell proliferation)

If all criteria are met, α2u-globulin nephropathy is considered the mechanism for the tumors and thus the tumor data not used for hazard assessment. Applying these criteria to rat bioassay data requires a detailed interpretation of the tumor and histopathology data from the NTP rat studies. But application of these 7 criteria is not cut and dry and there is considerable disagreement over the interpretation of them. And the strength of the association between α2u-globulin nephropathy and tumor incidence can he highly variable, suggesting other mechanism for tumor induction, even in the presence of α2u-globulin nephropathy.

Staff is proposing to develop a systematic approach to identify the key points in the nephropathy/tumor relationship to help focus the discussion with HEAC. The table below are the P1 and P2 PEL substances with the male and female rat kidney data from the NTP 2-year studies. Note that some are not inhalation studies – gavage or drinking water. α2u-globulin nephropathy occurs with some but not all of these substances. Chronic Peripheral Nephropathy (CPN) is a series of histopathological changes common in rat kidney that increase with age and which occurs in high incidence in controls. Evidence of a dose-response in CPN severity and incidence is important to establishing whether the chemical can have an effect on the kidney via α2u-globulin-nephropathy. In the table, numbers in parentheses are the incidence of CPN, not available for all studies. The rat tubule tumor (RTT) incidence is shown in the next column and factors to consider are whether there is a dose response and is it significant. The next columns are the conclusions about the α2u-globulin-nephropathy, CPN and carcinogenicity and findings on the genotoxicity and mode of action from various agencies. Genotoxicity is a key factor in the evaluation of the role of α2u-globulin-nephropathy in kidney tumors and mode of action is considered when α2u-globulin-nephropathy is ruled out or other tumor sites are considered for hazard assessment. Mechanism of action (MOA) can assess whether there is a threshold response and if the response is relevant to humans.

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The first two chemicals listed are for demonstration – limonene is the classic α2u-globulin nephropathy inducer and demonstrates the uniqueness of RTT to male rats – no RTT incidence was observed in females. And there is a dose response with CPN severity and incidence in males but nor females. 1,2,3-TCP is not an inducer of α2u-globulin nephropathy yet has the highest RTT in the table – 1,2,3 TCP is genotoxic and a multi-site carcinogen so the RTT are not due to α2u-globulin nephropathy. All the other chemicals in the table are on the PEL list and will be reviewed at some point. Staff proposes developing guideline to evaluate these criteria:

α2u-globulin (+/-) → CPN /RTT DOSE RESPONSE → OTHER TUMOR SITE(S) → GENOTOXICITY → MOA

The presence of α2u-globulin is a fairly straight – forward criteria to evaluate. CPN is a broad description of kidney damage so there are multiple measures of effect that can be evaluated for hazard assessment. The extent to which these measures are evaluated could be driven by the relationship between CPN severity and incidence. If that relationship is good, especially in female rates, that supports using the RTT data. This gives some confirmation that the chemical is having an effect on the kidney that is related to the tumor incidence. If the relationship is not good, other non-tumor endpoints of kidney damage can be considered for hazard assessment. Genotoxicity assessment can vary between convincing and equivocal results and may require a weight of evidence determination given the many different mutagenicity assays that are used.

This type of analysis of α2u-globulin nephropathy and RTT has been done by other agencies for TBA (IRIS) and MIBK (NTP). Applied to TBA, there appears to be no or weak association between α2u-globulin nephropathy and RTT in male and female rats. There are other data showing α2u-globulin nephropathy was found in this study and since there is virtually no dose response it could be concluded that these tumors are not determined α2u-globulin nephropathy. The question of genotoxicity is important here; IRIS discounts what genotoxicity data there are as being too variable to use for assessment and utilized other kidney endpoints (kidney weight, etc) for hazard assessment. OEHHA found a limited number of positive studies indicative that TBA is genotoxic. With this finding, RTT are relevant and OEHHA calculated a cancer risk slope and associated RfC. With MIBK, there is a similar pattern. There is no dose response in female rat CPN severity but there is in CPN incidence but no RTT. There appears to be a dose-response in male rat CPN and tumor incidence. In the draft MIBK summary, CPN in was used for derivation of a NOAEL and RfC for MIBK. In both cases, there were other tumors in rats or mice that could be evaluated.

The idea is to develop guidelines to direct staff effort into reviewing all the available analyses of the substances that cause RTT. The table is not complete for all substances but a comparison of CPN trends and RTT incidence might help prioritize what substances to work on. Some are not problematic; 1,2,3-TCP is genotoxic and a multi-site carcinogen so complete analysis would not be needed. HEAC has already completed this review. Other substances like furfuryl alcohol do not elicit α2u-globulin nephropathy but display CPN dose response and a weak male tumor response. FA is considered non-genotoxic but recently has been identified to produce DNA adducts (IARC, 2017 draft) so that classification needs to be updated.

The topic is for discussion.

Substance	Rat Study Type	CPN Mean Severity (Incidence) Control + Dose Groups	RTT Incidence, % (from seriel sections)	EPA/NTP/OEHHA/IARC/OTHER	Genotoxicity And Mode of Action
Limonene	M Gav F Gav	1.5 **1.8** **2.2** (82) (92) (98) NR NR NR (60) 66 58	0 **22.6** **25.8** 0 0 0	NTP: there was clear evidence *of* carcinogenic activity of d-limonene for male rats, as shown by increased incidences of tubular cell hyperplasia, adenomas, and adenocarcinomas of the kidney. No evidence ***of*** carcinogenic activity of d-limonene **for** female rats that received **300 or 600** mg/kg. No evidence of carcinogenic activity of d-limonene for male **B6C3F1** mice that received **250** or 500 mg/kg. No evidence *of **car***cinogenic activity of d-limonene for female **B6C3F1** mice that received 500**or** 1,000mg/kg. An increased severity of spontaneous nephropathy, increased incidences of linear mineralization of the renal medulla and papilla, and **hy**perplasia of the transitional epithelium of the renal papilla were present in dosed male rats.	NTP-347: d-Limonene was not mutagenic in four strains of *S. typhimurium* (TA98, TA100, TA1535, or TA1537), did not significantly increase the number of trifluorothymidine (Tft)-resistant cells in the mouse L5178Y/TK + / assay, and did not induce chromosomal aberrations or sister chromatid exchanges (SCEs) in cultured CHO cells. All assays were conducted in the presence and absence of exogenous metabolic activation.
123-TCP	M Gav	2.0 2.0 2.6 2.4	0 4 41 40	**IARC:** *is probably carcinogenic to humans (Group 2A)*	
Tert-butyl alcohol (TBA)	M DW F DW	3.0 3.1 3.1 **3.3** (49) (49) (50) (50) 1.6 **1.9** **2.3** **2.9** (48) (47) (48) (50)	2 6 8 6 (16) (26) **(38)** (26) 0 0 0 0	**IARC** – not evaluated **IRIS, draft 2017**: Based on mechanistic evidence indicating that an α2u-globulin-related process is operating in male rats, any kidney effects associated with α2u-globulin nephropathy are not considered relevant for human hazard identification. In addition, CPN played a role in the renal tubule nephropathy observed following TBA, and effects associated with such nephropathy are not considered relevant for human hazard identification. Although increases in severity (males and females) or incidence (females) of nephropathy were related to TBA exposure and could have arisen from chemical-specific processes independent from CPN, the association of these effects with CPN makes this measure less suitable for dose-response analysis, and therefore these effects were not considered for the derivation of reference values. The remaining effects (suppurative inflammation, transitional epithelial hyperplasia, and increased kidney weights) are considered the result of TBA exposure and relevant to human hazard characterization. These effects therefore are	**IRIS, 2017:** Overall, a limited database is available for understanding the role of tert-butanol-induced genotoxicity for mode of action and carcinogenicity. The database is limited in terms of either the array of genotoxicity tests conducted or the number of studies within the same type of test. In addition, the results are either conflicting or inconsistent. The test strains, solvents, or control for volatility used in certain studies are variable and could influence results. Furthermore, in some studies, the specificity of the methodology used has been challenged. Given the inconsistencies and limitations of the database in terms of the methodology used, number of studies in the overall database, coverage of studies across the genotoxicity battery, and the quality of the studies, the weight of evidence analysis is inconclusive. The available data do not inform a definitive conclusion on the

				suitable for consideration for dose-response analysis and derivation of reference values." **NTP**: some evidence of carcinogenic activity of TBA in male rats, no evidence of carcinogenic activity of TBA in female rats, equivocal evidence of carcinogenic activity of TBA in male mice, and some evidence of carcinogenic activity of TBA in female mice. **OEHHA**, **2017**: TBA genotoxicity data are mixed, but positive DNA damage and bacterial gene mutation studies for TBA suggest that TBA may cause oxidative DNA damage. It has been proposed that the NTP (1995) TBA carcinogenicity data may not be relevant to human cancer risk assessment because 1) the male rat kidney tumors are the result of TBA-induced α2u nephropathy, a pathological effect specific for male rats, and 2) the female mouse thyroid follicular cell tumors may be due to an effect on thyroid hormone levels, which would only occur above a threshold higher than the level of expected human exposures. However, the data pertaining to both these possibilities are insufficient to allow the determination that the NTP (1995) TBA carcinogenicity data are not relevant to human cancer risk assessment. Therefore, TBA should be considered to pose a potential cancer risk to humans.	genotoxicty of TBA and thus the potential genotoxic effects of TBA cannot be discounted. **OEHHA, 2017**: Positive genotoxicity data exist for TBA. TBA has been reported to cause DNA damage in human leukemia HL-60 cells using a Comet assay (Tang et al., 1997) and induce 8-OHdG DNA adducts and DNA damage measured via the Comet assay in Rat-1 cells (Sgambato et al., 2009). Additionally, TBA has been demonstrated to induce mutations in a Salmonella strain known to be sensitive to oxidative DNA damage (TA102) in the presence of rat liver S9 (Williams-Hill et al., 1999). These data indicate that TBA has genotoxic potential, and precludes a determination of non-genotoxicity.
MIBK	M Inh F Inh	2.**0** **2.6** **2.4** **3.1** (42) (45) (47) **(50)** 1.4 1.5 1.5 1.9 (19) **(35)** **(38)** **(44)**	0 2 4 8 (4) (8) (6) **(22)** 0 0 0 0	**IARC:** Sufficient evidence in animals/2B; non-genotoxic **IRIS:** developmental effects; no assessment of NTP study. **NTP 538**: CPN observed in almost all male rats including controls. There were significant increases in both the incidence (1800 ppm) and severity (all exposed groups). There were also significant increases in the incidence (all exposed groups) and severity (1800 ppm) of CPN in females. Lesions in males are suggestive of α2u-globulin nephropathy. Thus, the increase in the severity of the CPN in the present study, whether dependent on or independent of α2u-globulin, likely contributed to the increase in renal tubule tumors. Conversely, although there were increases in both the incidence and severity of CPN in female rats, the association between MIBK exposure and renal tumor induction was uncertain, and there was no evidence of renal tubule tumor induction in females. CPN in females was not due to α2u-globulin nephropathy, as female rats produce little if any hepatic α2u-globulin.	**NTP:** MIBK was tested for genotoxicity in the *Salmonella* mutagenicity assay, L5178Y/TK$^{+/-}$ mouse lymphoma assay, BALB/3T3 cell transformation assay, unscheduled DNA synthesis assay, and *in vivo* mouse bone marrow micronucleus assay (O'Donoghue *et al.*, 1988; Zeiger *et al*., 1992). Based on the observation of a marginal response only at the highest, cytotoxic concentration tested in the L5178Y/TK$^{+/-}$ mouse lymphoma assay, the lack of reproducibility of response in the BALB/3T3 cell transformation assay, and clearly negative results in the *Salmonella* mutagenicity assay, the unscheduled DNA synthesis assay, and the micronucleus assay, MIBK is not considered to be genotoxic.

Anthra-quinone	M oral F oral	2.2 3.1 3.0 3.0 1.2 1.4 1.3 1.5	2 **18** 10 6 0 **18** **16** **29**	**NTP:** Under the conditions of these 2-year feed studies, there was some evidence of carcinogenic activity of anthraquinone in male rats based on increased incidences of renal tubule adenoma and of transitional epithelial papillomas of the kidney and urinary bladder. Hepatocellular neoplasms may have been related to exposure to anthraquinone. There was clear evidence of carcinogenic activity of anthraquinone in female rats based on increased incidences of renal tubule neoplasms.	
chloroprene	M Inh	2.8 3.0 3.1 3.5	0 2 2 4 (2) **(16)** **(12)** **(16)**	IARC: *possibly carcinogenic to humans (Group 2B)* **NTP**: there is clear evidence of carcinogenicity in the F344/N rat and B6C3F1 mouse due to lifetime inhalation exposure to chloroprene. In rats, increased incidences of neoplastic lesions primarily occurred in the oral cavity (both sexes), lung (males only), kidney (both sexes), and mammary gland (females).	
Benzo-phenone	M oral	1.3 2.4 3.3 3.8	2 4 4 8 (4) (4) (14) **(16)**	**NTP-533:** there was some evidence of carcinogenic activity of in male F344/N rats based on increased incidences of renal tubule adenoma. **Michigan:** In the case of benzophenone, alpha 2u-g accumulation is not present in the male rat kidney. This is based on the absence of hyaline droplet accumulation in the kidney, a key indicator of alpha 2u-g accumulation (see NTP, 2006, Table A5, page 105). Therefore, the extrapolation of kidney dose-response data of kidney lesions (both cancer and non-cancer endpoints) in the male rat are appropriate to use to estimate human health risks from exposure to benzophenone. http://www.deq.state.mi.us/aps/downloads/ATSL/119-61-9/119-61-9_annual_ITSL_IRSL.pdf	**IARC**: Not mutagenic in ST TA98, 100 1535 or 1537 in presence or absence of metabolic activation systems. Did not increase frequency of micronucleated polychromatic erthyrocytes in bone marrow of mice treated with 200 – 500 mg/kg in the diet. Neither benzophenone nor two metabolites induced umu gene expression on ST TA 1535 in presence or absence of microsomes. Gene expression did occur when E. Coli membranes expressing P450 enzymes were added to the medium. Benzophenone showed no evidence of mutagenicity in vitro or in vivo. **NTP:** Benzophenone did not induce mutations in ST strains TA98, TA100, TA1535, or TA1537, with or without induced rat or hamster liver activation enzymes. Intra-peritoneal injections of 200 to 500 mg benzophenone did not induce micronuclei in bone marrow polychromatic erythrocytes (PCEs) of male mice. A small increase in the frequency of micronucleated PCEs was noted in the 400 mg/kg group,

					but the difference was not statistically significant. No increases in the frequencies of micronucleated normochromatic erythrocytes were seen in peripheral blood of male or female mice administered benzophenone for 14 weeks in feed over a concentration range of 1,250 to 20,000 ppm.
Furfuryl alcohol	M Inh F inh	2.9 2.9 3.1 3.7 1.9 1.9 1.9 2.4	2 2 4 0 (4) (4) (6) (8) 0 1 0 2 (0) (1) (2) (2)	IRIS, 1988: INH not assessed NTP: Under the conditions of these 2-year inhalation studies, there was some evidence of carcinogenic activity of furfuryl alcohol in male rats based on increased incidences of combined neoplasms of the nose. There was equivocal evidence of carcinogenic activity of furfuryl alcohol in female rats based on marginally increased incidences of neoplasms of the nose and renal tubule neoplasms. There was some evidence of carcinogenic activity of furfuryl alcohol in male mice based on increased incidences of renal tubule neoplasms. There was no evidence of carcinogenic activity of furfuryl alcohol in female mice exposed to 2, 8, or 32 ppm. Not genotoxic; equivocal IARC: 2B	**NTP:, 19##:** Furfuryl alcohol was not mutagenic in ST TA98, TA100, TA1535, or TA1537, with or without S9 activation enzymes. It did induce sister chromatid exchanges in cultured CHO cells in two trials conducted in the absence of S9. In the second trial without S9, significant cell cycle delay occurred at the highest dose requiring harvest of additional cells at a later time to provide sufficient cells for analysis. No induction of SCEs occurred following treatment with furfuryl alcohol in the presence of S9. Furfuryl alcohol did not induce Abs in CHO cells treated in the absence of S9, but in the presence of S9, an equivocal result was obtained. In the Abs test with S9, the first trial showed a clear dose-related increase in aberrations, with significant elevations seen at 500 and 1,000 μg/mL. Results of the second trial were negative, and the assay overall was determined to be equivocal. In vivo, no induction of SCEs, Abs, or micronuclei was noted in bone marrow cells of male mice after administration of furfuryl alcohol by intraperitoneal injection. In the Abs test, results of the initial 36-hour trial were positive (P=0.003). However, results of two additional 36-hour trials were negative and the assay was concluded to be negative overall. In conclusion, with the exception of the positive response observed in the SCE test in cultured CHO cells in vitro, no indication of genetic activity was seen with furfuryl alcohol.

					IARC (in prep, 2017) : Strong evidence suggests that furfuryl alcohol is metabolically activated via sulphate conjugation to electrophilic 2-sulphoxymethylfuran. Furfuryl alcohol-specific DNA adducts were found in non-tumour tissue of patients with lung cancer,13 in mice, and in bacteria expressing human sulphotransferase.
pyridine		2.3 2.3 2.5 2.6	2 2 4 **12** (4) (8) (12) **(20)**		
Cumene	MR-Inh	2.3 2.6 2.9 2.7	4 10 **16** 14		
Tetrahydro-furan	MR Inh	3.0 2.9 3.2 3.0	2 2 8 10		