## CLH Cumene Report Comments

- Toxicokinetics
    - The toxicokinetics section of the CLH report does not highlight the issue that in rats and mice some metabolic pathways for cumene are saturated at high doses, indicating non-linear toxicokinetics. As such, MOAs that are observed at saturating doses are not relevant to human health hazard assessment. This conclusion is supported by metabolism studies done in rats (Research Triangle Institute, 1989) demonstrating increasing concentrations of the recovered parent compound ($^{14}$C-cumene) in exhaled air at non-saturating exposures.
- Genotoxicity
    - While the CLH report concludes that cumene is not a genotoxicant, there is a discussion on limiting positive genotoxic findings that have been reported in the literature. This includes reports of increased K-*ras* and *p53* mutations in lung tissues of cumene treated animals when compared to controls.
        - While the report did indicate that the observed changes may be an effect rather than a cause of the multistage carcinogenic process, this observation is expected considering the observed alveolar metaplasia and hyperplasia; in such rapidly dividing tissues, mutations are more likely. Therefore, these observations are a resulting effect and not a cause.
        - The discussion on genotoxicity also includes mention of positive micronucleus assay results in rats via ip injection; there are several other *in vivo* cumene exposure studies that are negative for clastogenic/aneugenic activity that provide indirect evidence to support cumene as non-genotoxic (Research Triangle Institute, 1989) (Chen, Wegerski, Kramer, Thomas, & McDonald, 2011).
        - The CLH report also connects results from a FLARE assay to oxidative damage resulting from cumene exposure. The FLARE assay is a not a validated test method to assess genotoxicity and the indicated results are not reliable as they did not exhibit any association with dose-response or duration-response.
- Lung tumors
    - The report suggest that the CYP2F2-mediated MoA is not relevant to cumene due to the lack of cumene-induced lung cytotoxicity. Extensive CYP2F2-MoA studies done in structurally analogous chemicals (styrene and ethylbenzene) have indicated that lung cytotoxicity is not a required step in the CYP2F2 MoA (Cruzan, et al., 2018). These CYP2F2 MoA studies using structurally similar chemicals demonstrate a primary mitogen and not cytotoxicity as the driver for styrene-induced cell proliferation and hyperplasia (Cruzan, et al., 2018)

(Andersen, et al., 2017). These recent studies have refined the CYP2F2 MoA to establish that cytotoxicity is not required for cumene to act via this mechanism.

- Liver tumors
    - As pointed out in the CLH report, there were observed liver tumors in female B6C3F$_1$ mice at 500 ppm (NTP, 2009), the observed incidences were within the control range of the laboratory for studies using all routes of administration. Furthermore B6C3F$_1$ mice are known to have a high spontaneous background incidence of liver tumors, and therefore should be assessed for statistical significance using the Haseman Rule, which is used by several regulatory bodies (FDA, EPA, OECD). If this criteria is applied to the observed female mouse liver tumors, they are not significant and should not be used to establish cumene as a carcinogen.
    - The report also suggests the lack of data linking cumene to a CAR-like MoA. Studies done in structural analogues (ethylbenzene) support the applicability of the CAR mechanism to cumene (Yuan, Uwagawa, Okuno, Cohen, & Kaneko, 1997) (Sequeira, Eyer, Cawley, Nick, & Backes, 1992) (Serron, Diwivedi, & Backes, 2000). Adding to this evidence are observations of cumene induced changes, such as altered expression of CAR-associated genes and induction of CAR-associated cytochrome-P450 enzymes in response to cumene (Pfeffer, LeBaron, Battalora, Bomann, & Werner, 2018).
- Renal tumors
    - The CLH report highlights the occurrence of renal cell adenomas and carcinomas in male F344/N rats. When these tumors are evaluated using the Haseman Rule, (similar to applicability for liver tumors), they are considered common tumors and are not sufficient to meet statistical significance. Therefore, these observations should not be used to establish cumene as a carcinogen.
    - The report also indicates that cumene does not meet some of the IARC criteria for α2u-globulin mediated renal tumor development, specifically IARC criteria # 5 (Reversible binding of the chemical or metabolite to α2u-globulin). This criteria is considered additional supporting information and not essential to establish an α2u-globulin mediate MoA. Moreover, while there is not direct evidence of reversible binding, Chen et al (Chen, Wegerski, Kramer, Thomas, & McDonald, 2011) demonstrated increased radioactivity in male rat kidneys after oral doses of $^{12}$C-cumene compared to the lack of radioactivity in the kidneys of female mice; this demonstrates the rat-specific increases associated with the α2u-globulin MoA.
    - The report also indicated IARC criteria #2 (male specific nephropathy) and #6 (evidence of sustained cell proliferation) were not met for cumene. In reference to criteria #2, there was a slight increase in female rat nephropathy after cumene administration but was not statistically significant. Additionally, no dose-

related effects on renal tubule hyperplasia or renal pelvis transitional epithelium hyperplasia was observed in female rats, while these endpoints were significantly increased in male rats; this data fulfills the requirement for criteria #2. In reference to criteria #6, there is evidence of increased tubule hyperplasia in rat exposed to cumene for 2 years and increased PCNA staining (a marker of renal tubule cell proliferation) in rats after 13-weeks of cumene exposure (NTP, 2009).

- Nasal tumors
    - While the CLH report points to increased benign nasal tumors in rats of both sexes, the NTP report indicates these tumors were only observed at one low dose in female rats. The report also suggests potential progression of the benign nasal tumors to malignancy. Considering the absence of malignant neoplasms in the nasal tract of rat after 2 years of high dose cumene exposure, progression to malignancy is not likely (NTP, 2009). While the CLH report does not give a potential MoA for the nasal tumor development, for which rats are more susceptible (Jeffrey, Iatropoulos, & Williams, 2006) a CYP2F2-mediated MoA is likely and irrelevant to humans. This is supported by high CYP2F2 protein content in the olfactory epithelium when compared to other rat respiratory tissues. This information taken together with low levels of the CYP2F2 isoform found in human nasal epithelium demonstrate that the CYP2F2-mediated MoA acts in rats and not in humans.
- Other tumor types
    - The CLH report highlights other tumor types observed, including hemagiosarcoma, thyroid follicular tumors, and testicular interstitial cell tumors. The hemangiosarcoma and thyroid follicular tumor incidence were within historical control range and when evaluated using the widely-used Haseman Rule, they are not statistically significant. In reference to the testicular interstitial cell tumors, F344 rats have a high spontaneous background occurrence of this tumor type and several others, which led to NTPs decision to no longer use F344 rat. These observations demonstrate that this endpoint is unsuitable to establish carcinogenicity.
- Male reproductive toxicity
    - The CLH reported noted some observation from the NTP report on male reproductive toxicity, including questionable epididymal weight data in male mice. It is important to note that the whole epididymis and whole caudal epidydimal weight was not significantly different in high dose and control animals.

The CLH report submitted by the Danish Environmental Protection Agency was largely based on the tumor findings within the 2-year chronic cancer bioassay of cumene inhalation done by the U.S National Toxicology Program (NTP). Furthermore, the MoAs identified for cumene-

associated tumors are non-relevant to humans and several of the NTP results were analyzed in a statistically insufficient way. Taken together, the data presented within the CLH report fail to meet the criteria for classification as a carcinogen.

## References


Andersen, Cruzan, G., Black, M., Pendse, S., Dodd, D., Bus, J., & Sarang, S. (2017). Assessing molecular initiating events (MIEs), key events (KEs) and modulating factors (MFs) for styrene responses in mouse lungs using whole genome gene expression profiling following 1-day and multi-week exposures. . *Toxicological and Applied Pharmacology*, 28-40.

Chen, L., Wegerski, C., Kramer, D., Thomas, L., & McDonald, J. (2011). Disposition and metabolism of cumene in F344 rats and B6C3F1 mice. *Drug Metabolism and Disposition*, 498-509.

Cruzan, G., Bus, J., Andersen, M., Carlson, G., Banton, M., Sarang, S., & Waites, R. (2018). Based on an analysis of mode of action, styrene-induced mouse lung tumors are not a human cancer concern. *Regulatory Toxicology and Pharmacology*, 17-28.

Jeffrey, A., Iatropoulos, M., & Williams, G. (2006). Nasal cytotoxic and carcinogenic activities of systemically distributed organic chemicals. *Toxicologic Pathology*, 827-852.

NTP. (2009). *NTP Technical Report on the Toxicology and Carcinogenesis Studies of Cumene (CAS No. 98-82-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies).* Research Triangle Park, NC: National Toxicology Program.

Pfeffer, R., LeBaron, M., Battalora, M., Bomann, W., & Werner, C. (2018). Minimum datasets to estabslih a CAR-mediated mode of action for rodent liver tumors. *Regulatory Toxicology and Pharmacology*, 106-120.

Research Triangle Institute. (1989). *Metabolism, disposition and pharmacokinetics of cumene in F-344 rats following oral, i.v. administration or noise-only inhalation exposure.* RTI Srudy No 3543.

Sequeira, D., Eyer, C., Cawley, G., Nick, T., & Backes, W. (1992). Ethylbenzene-medicated induction of cytochrome P450 isozymes in male and female rats. . *Biochemical Pharmacology* , 1171-1182.

Serron, S., Diwivedi, N., & Backes, W. (2000). Ethylbenzene induces microsomal oxygen free radical generation: antibody-directed characterization of the responsible cytochrome P450 enzymes. . *Toxicology and Applied Pharmacology* , 305-311.

Yuan, W., Uwagawa, S., Okuno, Y., Cohen, S., & Kaneko, H. (1997). Time course for the modulation of hepatic cytochrome P450 after administration of ethylbenzene and its correlation with toluene metabolism. *Achives of Biochemistry and Biophysics*, 55-63.