



November 22, 2019

TO: The European Chemicals Agency
FROM: The American Chemistry Council Cumene Panel
RE: Harmonised classification and labelling public consultation on
Cumene

Dear ECHA,

Please see the attached technical comments on the CLH proposal for the classification of cumene as a carcinogen category 2, and the accompanying Final Interim Report (confidential) on the pilot liver study sponsored by the ACC Cumene Research Consortium.

Thank you,

Eileen Conneely

Eileen Conneely
Senior Director, Chemical Products and Technology
American Chemistry Council
On behalf of the ACC Cumene Panel



Technical Comments on the Evaluation on the CLH Proposal for the Classification of Cumene as a Carcinogen Category 2 (Suspected Human Carcinogen)

The competent authority proposing the Carcinogen Category 2 classification has primarily relied upon the 2009 U.S. National Toxicology Program (NTP)-sponsored chronic (lifetime) inhalation exposure studies conducted with mice and rats as the basis for such a classification. On the basis of increased incidences of respiratory epithelial adenomas observed in the nose and combined renal tubule adenomas or carcinomas in male F344/N rats, and increased incidences of alveolar/bronchial neoplasms in both male and female B6C3F₁ mice, the NTP concluded that cumene “exhibited clear evidence of carcinogenicity” in these test systems. Additional findings considered to be treatment-related included increased combined incidences of hepatocellular adenoma and carcinoma in female B6C3F₁ mice. Findings reported by NTP considered suggestive of cumene treatment included interstitial cell adenoma of the testis of male F344/N rats along with respiratory epithelial adenoma in the nose of female F344N/rats and splenic hemangiosarcomas and thyroid follicular cell adenoma in male B6C3F₁ mice.

To understand the potential relevance that these chronic toxicity findings may have to humans, a focused mode of action (MoA) research program supported by US and European cumene producers has been initiated under the direction of the ACC Cumene Research Consortium. The research program is designed to evaluate the relevance of the findings identified from the NTP bioassays, and includes the liver and lung of mice and the kidney of rats.

The liver tumor findings reported by NTP are discussed below, followed by a summary of the MoA mouse liver pilot study findings which provide compelling preliminary evidence that cumene treatment induces a nuclear receptor CAR-mediated process that drives hepatocellular adenoma and carcinoma formation. These findings indicate that this MoA is not relevant to cancer risk in humans.

Evaluation of Mouse Liver Tumors

As indicated in the CLH report, the strain of mouse (B6C3F₁) historically used in the conduct of NTP chronic bioassays is known to exhibit a high spontaneous background rate for hepatic tumors and consequently a number of regulatory authorities have urged caution when evaluating the statistical significance of these tumors when observed. In spite of acknowledging the high rate of spontaneous hepatic tumor incidence in this mouse strain, the CLH report concludes by stating there is “clear evidence for an exposure related causal effect in female mice.” When an incident rate adjustment is properly made for statistical evaluation, the hepatic tumor response no longer reaches statistical significance.

In a 3-month inhalation exposure study conducted by the NTP, male/female B6C3F₁ mice (10 mice/sex/group) were exposed to 62.5, 125, 250, 500, and 1000 ppm cumene vapor, 6 hours plus T₉₀ (12 minutes)/day, 5 days/week for 14 weeks (NTP, 2009). Two non-neoplastic lesions were reported in the liver of cumene-exposed female mice, as shown in Table 1 below.



Table 1: Incidences of non-neoplastic lesions in female mice in the 3-month inhalation study of cumene

Concentration	Chamber Control	62.5 ppm	125 ppm	250 ppm	500 ppm	1000 ppm
Liver (n)	10	10	10	10	10	10
Chronic Focal Inflammation	1 (1.0) [§]	10** (1.0)	10** (1.0)	9** (1.0)	7** (1.0)	2 (1.0)
Necrosis	4 (1.3)	0	0	0	2 (1.5)	0

** Statistically significantly different ($P \leq 0.01$) from the chamber control group

[§] Values in parentheses are average severity grade of lesions: 1=minimal, 2=mild, 3=moderate, 4=marked

Data in this table is extracted from Table 18 (p. 56) of the Cumene NTP report.

As noted in Table 1, minimal to mild grade necrosis was observed in 4 (of 10) control group mice, and only in 2 (of 10) in the 500 ppm exposure group, but was absent in any other exposure groups. In fact, the incidence of hepatic necrosis following 14 weeks of repeated exposure to 500 ppm cumene was 50% lower than that observed in chamber controls, with a similar severity grade. Overall, there was no clear evidence of a treatment-related effect of cumene on liver necrosis in female mice.

With respect to chronic focal hepatic inflammation, no concentration-response relationship was observed, and in fact a higher incidence occurred in the two lowest exposure groups (62.5 and 125 ppm) when compared with the two higher exposure groups (500 and 1000 ppm). Notably, no statistically significant difference in chronic focal hepatic inflammation in female mice exposed at the highest concentration of 1000 ppm cumene was observed when compared with chamber controls (1/10 chamber control and 2/10 at 1000 ppm). Moreover, for control and all cumene exposure groups, the average severity grade of focal hepatic inflammation was minimal (grade 1.0), and the severity grade did not progress with increasingly higher exposure concentrations. If, as assumed by NTP, this lesion was associated with cumene exposure, an increased incidence or an increase in severity of the chronic focal hepatic inflammatory response of animals exposed at comparable or higher exposure concentrations in longer term studies involving more frequent and extended exposure duration would be expected. For example, one would anticipate or predict observing similar incidences of inflammation/non-neoplastic lesions (with higher severity grades) and possibly the presence of pre-neoplastic foci, or even neoplastic lesions, following prolonged treatment-related hepatic inflammation. However, when the same mouse strain (50/sex/group) was exposed to 125, 250, or 500 ppm cumene vapor, 6 hours plus T₉₀ (12 minutes)/day, 5 days/week for 105 weeks (NTP, 2009), NTP reported no evidence of chronic focal hepatic inflammation or a treatment-related effect on eosinophilic foci (considered to be presumptive pre-neoplastic lesions by the NTP)¹ in the livers of exposed female mice. While the incidence of hepatocellular adenoma, and hepatocellular



adenoma and carcinoma (combined) showed positive trends for female mice, statistical significance was only achieved in the 500 ppm exposure group, but not in the lower exposure concentration groups where higher incidence rates of chronic focal hepatic inflammation were observed in the 90-day study. Overall, the lack of a concentration-response, in the absence of increasing lesion severity with increasing exposure concentration (minimal severity—average grade 1.0 for lesions across all treatment groups), and the absence of exacerbation to a more severe phenotype associated with increased exposure duration, make these data of limited utility in quantitative cancer risk assessment.

This finding of chronic focal hepatic inflammation was critically reviewed and disregarded as a significant observation or finding by two other regulatory agencies, the European Scientific Committee on Occupational Exposure Limits (SCOEL) and the German MAK-Commission. The German MAK-Commission referenced a 2011 letter from the NTP to the Commission in which the authors of the NTP study indicated that they did not consider the increase in chronic focal hepatic inflammation to be of any significance (Jahnke G et al., 2016). The SCOEL concluded that “*chronic inflammation reported in the liver of female mice in the 90-day study was of minimal grade and is a normal finding in the liver of mice and rats. Therefore, this liver lesion is seen by SCOEL to represent a normal background variation that does not carry much scientific weight*” (Bolt et al., 2016).

Mouse Liver Pilot Study Preliminary Findings Indicate a CAR-mediated MoA for Cumene

Female C57BL/6 mice were implanted with BrdU-filled minipumps and dosed by oral gavage for seven consecutive days with cumene (500 mg/kg, twice daily for a final total daily dose of 1,000 mg/kg). Samples were harvested for clinical chemistry, biochemical, gene expression (TaqMan®), and histopathology analyses. Statistically significant increases in liver weight, and liver to body weight ratio were observed in treated animals compared with controls, along with increases in levels of Cyp2b10, Cyp3a11, Cyp4a10, and Cyp4a14 mRNA, and EROD, PROD, and BROD activities. Notably, a concomitant increase in Total P450 levels was observed in treated animals compared with controls, coupled with a highly statistically significant increase in S-Phase labelling, a measure of cell proliferation in the absence of hepatic hypertrophy. These preliminary yet compelling findings strongly suggest that repeated oral treatment with cumene induces hepatic activation of CAR and possibly PXR in female C57BL/6 mice and is consistent with a phenobarbital-like MoA (Elcombe et al., 2014). Rodent hepatic carcinogens exhibiting a phenobarbital-like CAR-mediated MoA are not considered to be relevant to the induction of human hepatic tumorigenesis (Elcombe et al., 2014) and consequent



human liver cancer risk. The pilot study findings are to be used in the design of a 7-day inhalation exposure study of cumene with C57BL/6 mice. Additional work to be completed by the research program includes mouse lung and rat kidney MoA studies.

¹National Toxicology Program, NTP Nonneoplastic Lesion Atlas, Liver; *available at* https://ntp.niehs.nih.gov/nnl/hepatobiliary/liver/foci/liver-foci_508.pdf



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2. Bolt HM, Nielsen GD, Papameletiou C, Klein CL. (2016). 2-Phenylpropane (Cumene): Recommendation from the Scientific Committee on Occupational Exposure Limits (SCOEL/REC/029) ed., Directorate-General for Employment, Social Affairs and Inclusion, Scientific Committee on Occupational Exposure Limits.
3. Jahnke G, Hamann I, Laube B, Greim H, A H. (2016). Isopropyl benzene (cumene) [MAK Value Documentations, 2013] ed., The MAK-Collection for Occupational Health and Safety, Wiley-VCH Verlag GmbH & Co. KGaA.
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