May 9, 2007

Dr. Barbara S. Shane
National Toxicology Program (NTP) Executive Secretary
National Institute of Environmental Health Sciences
PO Box 12233 - MD A3-01
111 T.W. Alexander Dr.
Research Triangle Park, NC 27709

RE: NTP Draft Technical Report (TR-542): THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF CUMENE (CAS NO. 98-82-8) IN F344/N RATS AND B6C3F1 MICE.

Dear Dr. Shane:

The American Chemistry Council's Cumene Panel (the "Panel") offers the following comments on the NTP Draft Technical Report 542: The Toxicology and Carcinogenesis Studies of Cumene (CAS No. 98-82-8) in F344/N Rats and B6C3F1 Mice. The Panel appreciates the opportunity to submit these comments to the Technical Reports Review Subcommittee and is available to address any questions regarding these comments or to provide additional information regarding cumene. These comments include:

1. Typographical errors in the draft report;
2. Additional genotoxicity data on cumene not considered in the report;
3. Male rat kidney tumors and α-2u-globulin mode of action;
4. CYP2F2 mediated toxicity in mouse lungs and rat nasal tissue.

Dr. George Cruzan will represent the Panel at the upcoming Subcommittee meeting on May 16, 2007, will provide brief oral comments, and will be available to address any questions from Subcommittee members.

1. Typographical: The report presents the Methods and Results in a clear fashion. No errors in the reporting were found. We offer the following typographical corrections.

p. 22, structure of "phenyllactic acid" is not correct; it is lacking one carbon.

p. 90, Table lists exposure concentrations for females as 250, 500 and 1000; should be 125, 250 and 500 ppm.

p. 95, "A significant increase in the incidence of squamous metaplasia of the respiratory

epithelium occurred in 1,000 ppm females." Females were not exposed at 1000 ppm. Should be "500 ppm".

2. Genotoxicity: The report presents the genotoxicity studies performed by NTP and concluded "Cumene was not mutagenic in *S. typhimuriumstrain* TA97, TA98, TA100, or TA1535, when tested with and without liver S9 activation enzymes. Cumene induced small but significant increases in micronucleated polychromatic erythrocytes in bone marrow of male rats treated by intraperitoneal injection. In contrast, no increase in micronucleated erythrocytes was observed in peripheral blood of male or female mice exposed to cumene by inhalation for 3 months." The NTP TR-542 report evaluates the studies performed at NTP, but does not give a complete picture of the gentoxicity testing for cumene. As reported in the EPA IRIS Toxicology Review (EPA 1997), cumene was negative in two other Ames assays (Lawlor and Wagner, 1987; Florin et al., 1980). Cumene was negative in a series of HGPRT assays using Chinese hamster ovary cells (Yang, 1987) and a micronucleus assay in mice at up to 1000 mg/kg (Gulf Life Sciences Center, 1985b).

Cumene failed to induce significant rates of transformation in BALB/3T3 cells (without activation) at concentrations up to 500 g/mL (Putnam, 1987) but tested positive in an earlier cell transformation test also using BALB/3T3 cells, in which an increase in transformations was observed 60 g/mL (Gulf Oil, 1984a). One test for unscheduled deoxyribonucleic acid (DNA) synthesis (UDS) in rat primary hepatocytes, using exposures of up to 24 g/mL cumene (without activation), was negative (Curren, 1992), whereas results from an earlier test indicated UDS at doses of 16 and 32 g/mL cumene (Gulf Oil, 1984b). Those tests indicating positive mutagenic potential (Gulf Oil, 1984a,b) were considered equivocal by EPA (EPA IRIS 1997) because they were not reproducible. The metabolic pathways of this compound are generally well established and do not appear to involve any suspect reactive species. One *in vivo* mutagenicity test (micronucleus) did give a weakly positive result with a dose that produced mortality, although cumene gave negative results in a relatively complete battery of *in vitro* and *in vivo* mutagenicity tests, including gene mutation, chromosomal aberration, and primary DNA damage. The US EPA concluded that cumene was not genotoxic and not likely to be a genotoxic carcinogen (EPA IRIS 1997).

The NTP report should acknowledge the additional evidence that cumene is not genotoxic.

3. Male Rat Kidney Tumors: The Abstract section (p. 9) of the 2-Year rat study should include the mode of action data generated for kidney toxicity and tumors in male rats. It should include a statement that the male rat kidney toxicity and resulting tumors fit the α-2u-globulin mode of action (MOA). The investigators specifically studied male rat kidney toxicity and α-2u-globulin renal tumor MOA. Cumene caused increased hyaline droplets, which were demonstrated to contain α-2u-globulin; in addition, the total amount of α-2u-globulin in the kidney was increased and cumene-exposed male rats had the characteristic pathological lesions. The Discussion (p. 94) suggests but stops short of saying the male rat kidney tumors occurred by the α-2u MOA.

The report should be more conclusive and should better reflect the state that this MOA does not occur in humans and, therefore, these tumors are not relevant for human risk assessment.

4. CYP2F –Mediated Toxicity and Tumors: Based on studies with naphthalene, coumarin, styrene, and ethylbenzene, the lung effects of cumene in mice may likely be from metabolism by CYP2F2, as suggested by the draft NTP technical report. Similarly, since mouse nasal olfactory epithelium contains a large amount of CYP2F2 and studies have demonstrated that the nasal toxicity of styrene in mice is mediated by CYP2F2, it may well be that the nasal effects of cumene in mice is also caused by CYP2F2-generated metabolites. In rats nasal olfactory tissue also contains a large amount of CYP2F4, and nasal toxicity is seen in rats from exposure to naphthalene, styrene, cumene, and α-methylstyrene. While there is no experimental confirmation at this point, it may likely be that the nasal toxicity and tumors are mediated by CYP2F4-generated metabolites. The draft Report states "Biotransformation of cumene by CYP2F2 in mouse lung and nasal tissue and CYP2F4 in rat nasal tissue could lead to the observed species difference. (p. 96)." The human CYP2F1 is much less prevalent in these tissues and is much less effective at metabolizing these compounds than either 2F2 or 2F4.

The report should more fully acknowledge the CYP2F species differences between humans, rats, and mice and how tumors generated by CYP2F family enzymes in rats and mice are not likely relevant to human risk assessment.

Conclusion: The Panel would once again like to thank the NTP for its efforts on cumene and for the opportunity for provide comments on this draft technical report. The Panel believes that there is relevant and important genotoxicity and mode of action (MOA) information that could be added to or further discussed in the technical report document. In particular, there is MOA information on several of the key tumor findings from these studies, including male rat kidney tumors, male and female rat nasal tumors, male and female mice lung tumors and female mice liver tumors. Understanding these modes of action is critical for evaluating potential human cancer risks, especially since there is considerable evidence that the MOAs for these tumor types are not anticipated to be relevant to humans. Thus, while the studies may have met NTP criteria for clear evidence of carcinogenicity in animals, the MOAs indicate these are not relevant for human risk and the Panel strongly believes that NTP should more fully acknowledge and discuss these modes of action in the document.

Please contact me at (703) 741-5627 or at Andrew_Jaques@americanchemistry.com if the NTP or Subcommittee members have any questions regarding the Panel's comments.

Best Regards,

Andrew M. Jaques

Andrew M. Jaques, Director
ACC Cumene Panel