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# **RE:** *Public Comment Submitted by The Dow Chemical Company: Draft NTP RoC monograph on Cumene*

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### **Executive Summary**

The proposed listing of cumene in the National Toxicology Program 13<sup>th</sup> edition of the Report on Carcinogens (RoC) as "Reasonably anticipated to be a human carcinogen" is unwarranted.

Given the extensive closed system production and use of cumene in phenol/acetone manufacture (> 98% of use), not only are the numbers of potentially exposed workers extremely small (likely less < 500 total in production and use in the United States), but the exposures are also very limited (< 1 ppm). Likewise, the vast majority of general population exposure to cumene is attributable to mobile and point source emissions from fuels, primarily gasoline. Although the number of exposed individuals to such emission sources is likely very large, the resulting exposures to cumene are extremely low relative to exposure doses identified as producing toxicity and cancer in animals. In addition, and even more importantly, cumene is a natural component of fuels, and as such, it far more appropriate to evaluate the potential for cumene human carcinogenicity from fuel emissions in the context of toxicity tests of those substances. The animal toxicity and carcinogenicity of wholely-vaporized gasoline have been extensively studied and thus should serve as the primary if not the sole basis for any cancer evaluation of cumene emissions resulting from this exposure source, and which also contributes by far to the greatest numbers of potential human exposures. The draft monograph does not consider any of the

fuel toxicity evidence, which indicates a substantially lower concern for a potential human cancer hazard.

The draft NTP RoC monograph justifies the proposed listing primarily based on findings from rodent bioassays which identified male and female mouse lung tumors, female mouse liver tumors, and male rat kidney tumors. Because of the extremely limited exposure potential of cumene, both in numbers of exposed individuals and low levels of exposure, more detailed mode of action investigations exploring the human relevance of these high dose animal findings have not been a high research priority . Nonetheless, cumene-induced cancer findings parallel those of several close structural analogs for which more extensive mode of action investigations have been conducted (e.g., ethylbenzene, styrene, etc). Mode of action investigations of these compounds, including limited mode of action data available for cumene itself, suggest a high plausiblity that the three tumor findings of concern are likely mediated through non-genotoxic modes of actions of questionable qualitative and/or quantitative relevance to human cancer outcomes.

In contrast, the draft monograph outlines a significantly less biologically plausible alternative mode of action analysis in order to justify the relevance of animal tumorigenicity to potential human cancer hazard. Despite a robust body of evidence indicating cumene and its close structural analogs are not genotoxic, the RoC monograph evaluation has nonetheless postulated a genotoxic mode of action mediated through formation of low levels of epoxide and/or other reactive metabolite(s) of cumene. The monograph evaluation overlooks evidence from cumene structural analogs indicating that similar reactive metabolite formation from these substances is more plausibly associated with non-genotoxic, cytotoxic and other modes of action that are not relevant to adverse human health outcomes.

Thus, a consideration of the nature cumene tumorigenicity, the overall mode of action evidence available for cumene and its close structural analogs, and extremely limited numbers of exposed individuals coupled to extremely low exposures, do not justify a conclusion that cumene can be *"reasonably* anticipated" to be a human carcinogen.

The RoC Draft Monograph does not demonstrate, as required by the Section 301 of the Public Health Act, that "a significant number of persons residing in the United States are exposed" to cumene. Although cumene is indisputably a high production volume chemical, that production volume cannot be interpreted as directly indicating both high numbers and high levels of human exposures. As stated in the RoC monograph, greater than 98 percent of cumene production is dedicated to manufacture of phenol and acetone, and a substantial fraction of the remaining production used in the manufacture of acetophenone,  $\alpha$ -methylstyrene, diisopropylbenzene and dicumylperoxide. However, it important to note cumene is produced, stored and converted to phenol and acetone in close system processes for which only a very small number of workers experience potential exposures (EU, 2001). In the European Union, which has large scale cumene production and use patterns comparable to the United States, it has been estimated that only 110-200 workers are likely exposed (EU, 2001). The numbers of total exposed workers associated with large scale production of phenol and acetone from cumene is likely similar to that of cumene production (no data available). Although the NTP has never defined what constitutes "a significant number of [exposed] persons", the number of exposed individuals directly associated with cumene production and phenol/acetone manufacturing in the United States is likely less than 1000 and thus should not be regarded as a representing a "significant number" of exposed individuals, and particularly so given both the low potency and most plausible modes of action associated with cumene-induce animal tumors (see sections below).

Although consideration of exposure is not a direct requirement of RoC evaluations, potential adverse health concerns associated with the very small numbers of exposed workers should nonetheless be further tempered by data indicating that closed system production and manufacturing of cumene (> 98% of all total cumene) results in occupational exposures which are generally less than 1 ppm (EU, 2001). A 1 ppm worker exposure translates to a total worker systemic dose of approximately 0.35 mg/kg/day while a 1000 ppm 6 hr/day exposure to mice (liver tumorigenic dose) is approximately 1,843 mg/kg, resulting in a robust margin of exposure between a liver tumorigenic dose in mice and worker exposures of 5,266 (see appendix 1 for dose calculations). Although this representative margin of exposure estimation is based on comparison to the 1000 ppm rodent dose, margins of exposure estimates derived from other doses would be similarly high.

Beyond the extensive use of cumene in closed-loop production and manufacturing operations, potential occupational exposures may also be encountered through its presence as a component in hydrocarbon solvents, e.g., production of paints, offset printing operations, rubber goods manufacturing operations, and auto repair (spray painting). However, exposures in these uses are also generally less than 1 ppm

and the numbers of workers again are limited (EU, 2001). In addition, since cumene is a natural component of hydrocarbon solvents, its potential toxicity in such uses should ideally be evaluated as that associated with the entire solvent, and not cumene *per se*. The concentration of cumene of in such solvents is likely to be low. Another hydrocarbon mixture, gasoline, has in fact been extensively evaluated for long-term chronic health effects in animal bioassays (see below). No uses of cumene in consumer products have been reported, so this route of general population exposure is not expected (EU, 2001).

The monograph (Table 1-4) estimates that approximately 7,800 individuals live 0.5 mile, and 43,400 individuals within 1 mile, of cumene-emitting facilities. However, based on EUSES modeling of cumene air emission-related concentrations in Europe, it can be projected that most of these individuals would actually experience the majority of their exposures not from facility air emissions, but rather from mobile and other point sources associated with fuel and automotive emissions. EUSES modeling estimated that "local" air concentrations (exposures experienced no more than 100 meters from facility fencelines) translate to an estimated daily human dose of 0.11 mg/kg (EU, 2001). It is likely this dose would be experienced by a much smaller fraction of the total 7,800 individuals estimated to be living within a 0.5 mile radius of production facilities in the United States, but even then, the margin of exposure for such 100 meter exposures to the 1000 mouse liver tumorigenic dose is 16,754. The EUSES model also projects that almost all individuals living within 1 mile of production facilities would experience exposures comparable to that modeled for "regional", or urban, exposures that are largely if not entirely attributable to fuel-related emissions. The estimated cumene human dose associated with regional exposures is  $1.45 \times 10^{-5}$  mg/kg/day (typically representing up to 20 million people). This regional dose translates to extremely large margins of exposure of 127,000,000 relative to the 1000 ppm mouse dose and 1,587,500 to the 125 ppm mouse exposure (the lowest dose tested). In addition to EUSES modeling, the monograph states that overall average urban air concentrations are 14.7  $\mu$ g/m<sup>3</sup> (the monograph is not clear how this value was developed or whether it represents only United States). Converting this value to an estimated daily human dose of cumene of 2.1  $\mu$ g/kg day (assumes 20 m<sup>3</sup> inhaled per 70 kg person per day and 0.5 respiratory retention'; Appendix 1), the margin of exposure relative to a 1000 ppm mouse exposure is > 877,000. These extremely large margins of exposures representing the vast majority (significant number) of this potential exposed human population strongly suggest cumene would not be a "reasonable" contributor to human cancer outcomes at these exposure levels.

Emissions from fuel and automotive sources are the primary contributor to regional cumene air concentrations (EU, 2001). However, because cumene is a natural constituent of fuels and petroleumbased substances, any potential risks (and by inference, hazards) should be preferentially evaluated in the context of cumene's presence in those materials, and not just from cumene per se (EU, 2001). Importantly, the carcinogenicity and mode of action of wholely-vaporized gasoline has in fact been extensively investigated in rodent studies (reviewed in Swenberg, 1993). Male rat specific renal tumors represent the primary cancer finding and that response has been robustly shown to be attributable an  $\alpha$ -2u-globulin mode of action primarily associated with the isooctane fraction of gasoline, and, is not regarded as relevant to humans (Swenberg, 1993). Gasoline exposure also increased the incidence of female mouse liver tumors at the highest dose tested, approximately 2000 ppm; the mode of action of that response has been postulated to be due to anti-estrogenic activity associated with increased hepatic P450 metabolism stimulated by a variety of gasoline components. However, cumene is not suspected as the gasoline component responsible for this response in that the low levels of cumene in gasoline do not make it a reasonable candidate contributor to gasoline toxicity. Thus, given that the vast majority (significant numbers) of human exposure to cumene is directly tied to fuel and associated automotive emissions, the gasoline rodent cancer bioassay assays are the most appropriate studies on which to evaluate potential general population cancer hazards to cumene. Those findings strongly suggest that cumene originating from such hydrocarbon emission sources cannot be reasonably considered a cancer hazard.

Beyond the lack of exposure relevance, the cumene rodent cancer findings used as the rationale supporting the proposed RoC listing, mouse lung and liver tumors and male rat kidney tumors, are plausibly mediated through modes of actions of questionable quantitative and/or qualitative relevance to human cancer hazards.

As noted above, because of the extremely limited exposure potential for cumene, detailed mode of action investigations are lacking for cumene. However, all three of the key target organ responses identified as the basis for the proposed RoC listing are closely associated with animal bioassay responses for which mode of action investigations have challenged their relevance to human cancer outcomes. In this regard, more detailed mode of action investigations with close structural analogs to cumene, and for cumene itself, indicate that the dose-dependent carcinogenicity of cumene most plausibly results

from non-genotoxic actions (detailed in sections below) as follows : 1) male rat specific kidney cancer mediated through  $\alpha$ -2u-globulin (not relevant to humans); 2) mouse lung toxicity mediated through mouse lung specific metabolism by CYP2F2 (not quantitatively and more likely qualitatively relevant to humans); and 3) enhanced mouse liver tumors mediated through a phenobarbital-like enzyme induction (not regarded a relevant to humans).

### Male rat specific kidney tumors mediated through $\alpha$ -2u-globulin

As detailed in the RoC monograph, the evidence supporting this mode is strongly supported by evidence derived directly from cumene specific studies. Examination of the data from cumene kidney toxicity response investigations are entirely consistent with the conclusion that the criteria required to classify cumene as reasonably operating by an  $\alpha$ -2u-globulin mode of action are generally fulfilled (see Table 5-1, monograph). Although the draft monograph regards it as questionable as to whether the criteria are fulfilled for 4 of the 7 requirements, a close examination of the overall data indicates that most of the NTP's concerns regarding the four unfulfilled criteria of concern are not substantive. Thus, it is highly likely cumene produces male rat kidney tumors by an  $\alpha$ -2u-globulin mode of action.

The monograph describes data supporting the conclusion that criteria 3, 4 and 7 are fulfilled with existing information from cumene studies. This comment concurs with that conclusion.

Regarding criteria 1, the monograph states that the genotoxicity evidence for cumene is equivocal and thus a genotoxic mode of action cannot be ruled out. However, an evidence-based analysis of the of the cumene genotoxicity data, when coupled to additional genotoxicity information available from its close structural analogs, provides a compelling case that cumene is not genotoxic (see the consideration of alternative modes of action section below). Thus, critieria 1 should be reasonably interpreted as being fulfilled – cumene is not genotoxic.

NTP asserts that criteria 2, male rat specificity for nephropathy and tumorigenicity, is not fulfilled. However, the animal bioassay data clearly indicate that tumorigenicity is restricted to male rats (Table 4-3), and that the cancer findings are well correlated to the characteristic non-cancer kidney toxicity associated with an  $\alpha$ -2u-globulin mode of action (Tables 5-2 and 5-3). The NTP conclusion that this criteria is not fulfilled due to a lack of male rat specificity for nephropathy rests on very weak supporting data. The data presented in Table 5-3 indicate that nephropathy increase in female is highly questionable in that only a slight, non-statistically significant increase in the incidence of nephropathy was apparent above an already high background incidence of this lesion in female rats. Thus, it is reasonable to conclude that criteria 2 is also fulfilled – kidney toxicity and tumorigenicity are male rat specific.

Although evidence for criteria 5, reversible binding of cumene and/or its metabolite to  $\alpha$ -2u-globulin, is not fulfilled by direct evidence, it is not unreasonable to postulate that such binding would be entirely expected and consistent with evidence available from structural analogs. As reported in the monograph, Chen et al. (2011) noted increased tissue retention of cumene-derived radioactivity in rat kidneys relative to male and female mice that was regarded as consistent with binding to  $\alpha$ -2u-globulin. In addition, the primary metabolite of cumene identified in rats is 2-phenyl-2-propanol. This tertiary alcohol cumene metabolite is a structural analog to the tertiary alcohol metabolite of isooctane, 2,4,4trimethyl-2-pentanol, which has been identified as a key metabolite driver of the  $\alpha$ -2u-globulin (reviewed in Swenberg, 1993). Thus, it is reasonable to expect the cumene metabolite 2-phenyl-2propanol is very likely capable of reversible binding to  $\alpha$ -2u-globulin. Even absent direct evidence of binding, however, the overall profile of kidney toxicity and tumorigenicity is entirely consistent with this mode of action.

Criteria number 6, evidence of sustained cell proliferation of the renal cortex, also has not been characterized in detail. However, the monograph notes that PCNA staining of kidneys from rats exposed to cumene for 14 weeks found that the mean numbers of proximal tubule cells in S-phase were significantly increased in the two highest treatment groups (500 and 1000 ppm) although the numbers of cell labeled and the labeling index were not altered. Absent a more robust method for assessing cell proliferation such as BrdU incorporation, these data nonetheless indicate the likelihood that cumene stimulated cell turnover in the target cells for the  $\alpha$ -2u-globulin mode of action, renal proximal tubule cells. This conclusion is also consistent with robust evidence that both unleaded gasoline and the 2,4,4-trimethyl-2-pentanol metabolite of isooctane stimulate sustained renal cell proliferation (Swenberg, 1993). Even in the absence of direct evidence, it is fully reasonable that fulfillment of criteria 6 probable and would be expected on further investigation.

In sum, an integrated interpretation of the data indicates it is highly plausible that cumene induced male rat kidney toxicity is mediated through an  $\alpha$ -2u-globulin mode of action that is not regarded as relevant as a human hazard or risk.

Mouse lung tumors are mediated through mouse lung specific metabolism by CYP2F2 that induces a nongenotoxic and cytotoxic, cell-regenerative mode of action that is quantitatively and likely qualitatively irrelevant to human lung toxicity and cancer.

Cruzan et al. (2009) have developed a unified mode of action hypothesis accounting for mouse specific lung toxicity and tumorigenicity observed in a series of close structural analogs including cumene. The hypothesis proposes that lung tumorigenicity is mediated through mouse specific lung metabolism of aromatic structural analogs by CYP2F2 to ring-oxidized, non-genotoxic and cytotoxic metabolite(s). The aromatic-oriented CYP2F2-mediated metabolism contrasts with metabolism of these substances by CYP2E1, which is a major contributor of the non-aromatic moieties of this class of compounds, e.g., vinyl substituent for styrene, ethyl substituent for ethylbenzene, and likely isopropyl substituent for cumene. CYP2E1 alkyl group metabolism, i.e., vinyl and/or ethyl/isopropyl metabolism, also represents the primary metabolic pathway for all the compounds in this series. However, reactive and cytotoxic ringoxidized metabolites associated with CYP2F2 metabolism, which is localized to lung target cells (terminal bronchiole Clara cells enriched in P450 enzymes), induces localized cell toxicity followed by regenerative repair that ultimately transitions to an increased incidence of late developing lung tumors. All chemicals in this class, including cumene, exhibit no evidence of lung toxicity and tumors in rats, which is consistent with observation that rats have both lower population of Clara cells and associated activity of the rat CYP2F2 ortholog, CYP2F4. Importantly, the human ortholog to CYP2F2, CYP2F1, is expressed a much lower levels compared to rodents, suggesting chemicals of this class are unlikely to present a quantitative, and more likely a qualitative, toxicity hazard to humans.

Evidence that cumene belongs in the CYP2F2-mediated mode of action class is indicated by the close parallel between its toxicity and tumorigenicity pattern to other more well studied and closely structurally related members of the class. As a foundational observation, the comparative rat and mouse lung toxicity of cumene is entirely consistent with this mode of action, i.e, the presence of lung toxicity and tumors in mice and an absence of both in rats. Although specific mode of action is lacking for cumene, a more extensive series of mode of action investigations is available for ethylbenzene, an extremely close structural analog to cumene and which also exhibits mouse, but not rat lung, toxicity and tumors (reviewed in Cruzan et al., 2009). Ethylbenzene is a non-genotoxic agent whose metabolism also closely parallels that of cumene. The primary oxidative metabolite of ethylbenzene is 1phenylethanol, which is the structural analog of the primary oxidative metabolite of cumene, 2-phenyl-2-propanol (Chen et al., 2010). It is well established that alkyl-oxidized metabolites of ethylbenzene are not responsible for lung toxicity and tumors, however, in that 1-phenyethanol did not exhibit either mouse or rat lung toxicity and tumors in chronic oral gavage bioassays (NTP, 1990; reviewed in Cruzan et al., 2009). Thus, the ethylbenzene findings strongly suggest that alkyl-oxidized metabolites were not critical to the lung toxicity mode of action of this agent and would be expected to similarly apply to the primary oxidative metabolite of cumene.

Ethylbenzene also increased cell replication (measured by BrdU incorporation) in the terminal bronchioles of mice exposed for either 1 or 4 weeks to a lung tumorigenic dose of 750 ppm but not a non-tumorigenic dose of 75 ppm (Stott et al., 2003). Also consistent with the CYP2F2 mode of action hypothesis, more recent studies have shown that formation of reactive ring-oxidized metabolites of ethylbenzene, although substantially less than alkyl-oxidized metabolite formation, was greater in mouse lung microsomes relative to rat lung and was very significantly reduced in human lung microsomes (Saghir et al., 2009, 2010). The greater ratio of alkyl- to ring-oxidized metabolite generation noted for ethylbenzene is similar to what has also seen in *in vivo* metabolism studies of cumene (Chen et al., 2011).

Chen et al., (2011) found that cumene undergoes an apparent small amount of alkyl group dehydration in mice with resultant formation of α-methylstyrene. The monograph speculates that α-methylstyrene could be subsequently metabolized to reactive and potentially genotoxic α-methylstyrene-oxide (alkylepoxide), thus describing a putative mode of action for lung toxicity and tumors. However, this hypothesis is strongly refuted by recent studies examining the modes of action of lung toxicity of both styrene and styrene oxide in knockout mice lacking CYP2F2 activity and in humanized mice in which the human CYP2F1 ortholog of CYP2F2 has been inserted into the CYP2F2 knockout mice (Cruzan et al., 2009, 2013). Both styrene and styrene oxide are close structural analogs to α-methylstyrene and its oxide metabolite, and as such, both styrene mouse lung toxicity and tumors have been hypothesized as being mediated through formation of styrene oxide from styrene (NTP, 2011; note: the 2011 NTP RoC listing of styrene is currently under review by the National Academy of Sciences National Research Council). However, while both styrene and styrene oxide are lung toxic in wild-type mice (measured as increased cell replication by BrdU), both were completely inactive in CYP2F2 knockout mice (Cruzan et al., 2009). The absence of styrene oxide lung toxicity in knockout mice further affirms the hypothesis that styrene oxide is not the proximate lung toxic metabolite, and by read-across inference to cumene,  $\alpha$ -methylstyrene-oxide as well. Even more importantly, however, both styrene and styrene oxide also were inactive in CYP2F1 humanized mice, strongly supporting the conclusion that mouse lung tumors in this series of structurally related compounds are unlikely to be quantitatively, and more likely qualitatively relevant to human lung toxicity (Cruzan et al., 2013). Thus, the cluster of studies with close structural analogs of cumene plausibly support the hypothesis that the mode of action of mouse lung toxicity of cumene is mediated through mouse lung specific metabolism to non-genotoxic and cytotoxic ring-oxidized cytotoxic metabolites by CYP2F2 that is not relevant to humans.

## *Cumene mouse liver tumors are plausibly mediated through a phenobarbital-like liver enzyme induction mode of action that is not a quantitatively relevant cancer hazard to humans*

The incidence of cumene mouse liver tumors was significantly elevated only in females exposed to the highest test concentration, and occurred on top of a typical high background tumor incidence in female mouse liver (55.6% in the cumene study). Cumene-induced tumors were associated with the hallmark features of a phenobarbital-like enzyme induction mode of action that has been qualified as to its kinetic and quantitative relevance to human tumor outcomes (Holsapple et al, 2006). Those features include: non-genotoxicity (see below); rapid and early onset of liver enlargement; evidence of saturated metabolism leading compensatory induction of CYP2B1/2B2; association of tumors with eosinophilic foci (a biomarker of this mode of action); and late developing tumors. Both these features and several additional features of a phenobarbital-like mode of action for cumene are further informed by read-across information from close structural analogs ethylbenzene, 1-phenylethanol, and n-propylbenzene: increased mitogenic cell replication at tumorigenic doses; evidence of saturated P450 metabolism at tumorigenic doses; confimed induction of CYP2B related enzymes; and increased liver hypertrophy.

Although detailed information is generally lacking to explain the mode of action of cumene mouse liver tumors, the nature of its tumorigenic response and other mode of action information collected from close structural analogs indicates a high probability that cumene belongs to the class of substances operating by a phenobarbital-like mode of action. As summarized in the monograph, the cumene liver response is characterized by liver enlargement, the presence of eosinophilic foci, specificity of liver toxicity to mice, and late developing tumors layered on a high background tumor incidence. Cumene metabolism is also likely saturated at both the two top test concentration in the cumene bioassay (500 and 1000 ppm; equivalent to 921 and 1,843 mg/kg/day; see Appendix 1) in that Chen et al (2011) found clear evidence of metabolic saturation in mice orally dosed with 1000 mg/kg of cumene (nonlinear increase in exhaled volatile organic compounds).

Saturation of cumene metabolism and induction of compensatory CYP2B1/CYP2B2 can also be inferred by analogy to structural analogs bracketing the cumene structure. The primary metabolism of ethylbenzene to 1-phenylethanol mediated by CYP2E1 is saturated at its liver tumorigenic dose of 750 ppm (reviewed in VCCEP, 2007). In addition, liver enzyme activity reflecting CYP2B1 induction was statistically increased after 4 weeks of treatment at 750 ppm (Stott et al., 2003). Backes et al. (1993) have also reported that n-propylbenzene was equally potent to ethylbenzene in inducing CYP2B1/2B2 in rats.

Finally, the toxicity profile of ethylbenzene parallels that cumene, i.e., liver enlargement, increase in late developing female mouse liver tumors, and presence of eosinophilic foci at tumorigenic doses. Importantly, the primary alkyl-oxidized metabolite of ethylbenzene, 1-phenylethanol, was not hepatotoxic or tumorigenic, suggesting that interaction with P450 metabolism (saturation of CYP2E1 followed by CYP2B1/2B2 induction) was a key element in the mode of action of liver tumorigenicity. By analogy, the primary oxidized metabolic of cumene, 2-phenyl-2-propanol, would also not be expected to hepatotoxic.

Thus, although detailed mode of action information is not available to explain cumene liver toxicity, the profile of cumene liver toxicity and tumorigenicity and associated mode of action information available for cumene itself and structurally related compounds make it highly plausible that cumene liver injury is consistent with a phenobarbital-like mode of action that is quantitatively not relevant to humans.

The alternative mode of action mediated through formation of a genotoxic epoxide metabolite of  $\alpha$ methylstyrene postulated in the monograph is not highly plausible. The genotoxic potential of cumene has been evaluated in a series of *in vitro* and *in vivo* traditional genotoxicity assays. Although the monograph concludes the overall evidence for the genotoxicity of cumene is "equivocal", such a finding is inconsistent not only with the data available for cumene itself but also for that of its close structural analogs. For cumene, this conclusion is best summarized in a publication authored by NIEHS scientists (Hong et al., 2008) in which it was stated:

"Cumene is not genotoxic, which was demonstrated by several studies involving bacterial and mammalian cells in culture and in in vitro studies involving mice and rats (NTP 1996; National Library of Medicine Hazardous Substance Data Bank 2003; US Environmental Protection Agency 1997). In vitro cell transformation assays using BALB/3T3 mouse embryo cells and unscheduled DNA synthesis assays using rat primary hepatocytes yielded conflicting results regarding a cumene effect that were not reproducible. Cumene was weakly positive with no clear dose response for the induction of micronuclei in rat bone marrow at doses ranging from 78 to 2500 mg/kg intraperitoneally (NTP 1996)."

The genotoxicity profile of cumene structural analogs ethylbenzene, styrene and other substances are also generally negative (reviewed in Cruzan et al., 2009) and are not consistent with a genotoxic mode of action. Parallel to the postulated formation of  $\alpha$ -methylstyrene-oxide from cumene, styrene oxide has been suggested as the key genotoxic metabolite of styrene (NTP, 2011). However, not only is the observation that styrene only produced tumors in single species in a single target organ not consistent with a tumorigenicity profile of genotoxic substances, but also both the *in vitro* and *in vivo* genotoxicity of styrene is not consistent with a genotoxic mode of action as well. Genotoxicity of styrene has also been postulated based on the demonstrated formation of styrene oxide protein and DNA adducts. However, it is highly unlikely these DNA adducts are responsible for mutational outcomes in that the adduct levels are extremely low and do not correlate with the target tissue (mouse lung) or the target species (adduct levels are similar or higher in rats, a non-responding species; Cruzan et al., 2009). By read-across inference, DNA adducts from  $\alpha$ -methylstyrene-oxide are also unlikely to influence the genotoxicity of cumene in that SO is a major metabolite of styrene while  $\alpha$ -methylstyrene-oxide is a minor metabolite of cumene. Finally, these observations are further supported by recent mode of action investigations that strongly indicate that styrene oxide (and by inference,  $\alpha$ -methylstyrene-oxide) is not the proximate lung toxic metabolite of styrene (Cruzan et al., 2012; 2013).

The monograph conclusion regarding the genotoxicity of cumene appears to be heavily influenced by recent work that identified an increase incidence of k-*ras* and p53 mutations in cumene tumors relative to spontaneous control lung tumors examined at terminal sacrifice. Because these assessments were made in tumors at terminal sacrifice, the mutational spectra as investigated do not reveal early genotoxic actions, and particularly those that might be directly attributed to cumene genotoxicity. The

authors' themselves note that the source of mutations were not identified and could well be the result of secondary influences such as increased oxidative stress. Given the highly plausible non-genotoxic, cytotoxic and cell replicative mode of action associated with close structural analogs of cumene (see above), it would be expected that a variety of biological responses associated with cytotoxicity, including oxidative stress, would be capable of inducing such end-stage mutations in late sampled cumene tumors. Thus, absent more thorough dose and time response investigations, as well as application of this methodology to other non-genotoxic substances, the implications of these findings for informing the direct genotoxic potential of cumene is unknown.

Thus, the genotoxic mode of action mediated through formation of low levels of  $\alpha$ -methylstyrene-oxide hypothesized in the monograph is far less plausible than the non-genotoxic, cytotoxic and/or enzyme-inducing modes of action supported by the body of evidence available for both cumene and several close structural analogs and their metabolites.

#### Conclusions

The listing of a chemical in the Report on Carcinogens as "reasonably anticipated to be a human carcinogen" was intended Congress to reflect substances for which a true concern for human cancer was "reasonable". Thus, during the legislative discourse associated with creation of the RoC, Congress was clear that substances that have only suggestive evidence of carcinogenicity for humans should not be listed at all. That is, the RoC was not directed to list "possible" or "suspected" carcinogens. The mandate from Congress was clear on this point: "the phrase 'suspected carcinogens' [was replaced] with 'substances . . . reasonably anticipated to be carcinogens,' in order to make it absolutely clear in the statute that there must be reasonable grounds for designating a substance as a putative carcinogen." Joint House-Senate Comparative Summary). An overall evidence based assessment of the both exposure and toxicity profile of cumene, and inclusive of an extensive body of information on close structural analogs of cumene, does not fulfill the expectation that cumene should be "reasonably anticipated to be a human carcinogen" hazard.

The most plausible modes of action indicate that cumene induces cancers that are either not qualitatively relevant to humans, or at worst are associated with very high-dose specific modes of action that are not quantitatively relevant to human cancer hazard. In addition, the vast majority of cumene production and use in phenol and acetone production (>98%) is associated with large scale closed systems operations. These applications result not only in extremely limited numbers of both potentially exposed workers and individuals living in close proximity to production and manufacturing facilities, but also in extremely low exposures relative to tumor-inducing doses in animal studies. The largest potential for cumene exposure is associated by far with emissions of cumene as a natural component of petroleum fuels and other derived hydrocarbon substances. Importantly, however, the human cancer hazard and risk of those hydrocarbon substances substances is preferentially evaluated from extensive animal studies conducted on wholely-vaporized gasoline and not directly from cumene studies. Those studies identified male rat kidney toxicity as the primary endpoint of concern, and subsequent mode of action studies conclusively identified an  $\alpha$ -2u-globulin mode of action attributable the isooctane fraction of gasoline. Thus, the combination of toxicity, mode of action, and exposure information available for **cumene and its close structural analogs fall well short of justifying a listing of cumene as** "*reasonably* **(emphasis added) anticipated to be a human carcinogen".** 

### **References:**

Backes, WL, Sequeira, DJ, Cawley, GF, Eyer, CS. Relationship between hydrocarbon structure and induction of P450: effects on protein levels and enzyme activities. Xenobiotica 23: 1353-1366, 1993

Chen, L-J, Wegerski, C, Kramer, D, Thomas, L, McDonald, J, Dix, K, Sanders, J. Disposition and metabolism of cumene in F344 rats and B6C3F1 mice. Drug Metabol Disp. 39: 498-509, 2011.

Cruzan, G, Bus, J, Banton, M, Gingell, R, Carlson, G. Mouse specific lung tumors from CYP2F2-mediated metabolism: And endpoint/toxic response where data from multiple chemicals converge to support a mode of action. Reg. Toxicol. Pharmacol. 55: 205-218, 2009

Cruzan, G, Bus, J, Hotchkiss, J, Harkema, J, Banton, M, Sarang, S. CYP2F2-generated metabolites, not styrene oxide, are a key event mediating the mode of action of styrene-induced mouse lung tumors. Reg. Toxicol. Pharmacol. 62: 214-220, 2012.

Cruzan, G, Bus, J. Hotchkiss, J, Sura, R, Moore, C, Yost, G, Banton, M, Sarang, S. Studies of styrene, styrene oxide and 4-hydroxystyrene toxicity in CYP2f2 knockout and CYP2F1 humanized mice support lack of human relevance for mouse lung tumors. Reg. Toxicol. Pharmacol., in press, 2013.

EU. Risk Assessment Report: Cumene. 2001.

Holsapple, M, Pitot, H, Cohen, S, Boobis, A, Klaunig, J, Pastoor, T, Dellarco, V, Dragan, Y. Mode of action in relevance of rodent liver tumors to human cancer risk. Tox. Sci. 89: 51-56, 2006.

Joint House-Senate Comparative Summary. Explanation of Title II of H.R. 12460 and H.R. 12347, as Reported by the Committee on Interstate and Foreign Commerce, the Senate Bill, S. 2450, and the

House Amendment in the Nature of a Substitute, 124 CONG. REC. H38657 (1978) (statement of Rep. Rogers).

NTP. Toxicology and carcinogenesis studies of  $\alpha$ -methylbenzyl alcohol. NTP Technical Report 369, 1990.

NTP. Styrene. Report on Carcinogens. 12<sup>th</sup> Edition, pp. 383-390, 2011.

Saghir, S, Rick, D, McClymont, E., Zhang, F, Bartels, M, Bus, J. Mechanism of ethylbenzene-induced mouse-specific lung tumors: Metabolism of ethylbenzene by rat, mouse, and human liver and lung microsomes. Tox. Sci., 107: 352-366, 2009.

Saghir, S, Zhang, F, Rick, D, Kan, L, Bus, J, Bartels, M. In vitro metabolism and covalent binding of ethylbenzene to microsomal protein as a possible mechanism of ethylbenzene-induced mouse lung tumorigenesis. Reg. Toxicol. Pharmacol. 57: 129-135, 2010.

Stott, W, Johnson, K, Bahnemann, R, Day, S, McGuirk, R. Evaluation of potential modes of actions of inhaled ethylbenzene in rats and mice. Tox. Sci. 71: 53-66, 2003.

Swenberg, J.  $\alpha$ 2u-Globulin nephropathy: Review of the cellular and molecular mechanisms involved and their implications for human risk assessment. Env. Hlth. Perspect. 101 (supplement 6): 39-44, 1993

Hong, H-H, Ton, T-V, Kim, Y, Wakamatsu, N, Clayton, N, Chan, P-C, Sill, R, Lahousse, S. Genetic alterations in K-ras and p53 cancer genes in lung neoplasms from B6C3F1 mice exposed to cumene. Tox. Path. 36: 720-726, 2008.

VCCEP. Voluntary Children's Chemical Evaluation Program. Tier 1 pilot submission for ethylbenzene. (submitted by various industry sponsors), 2007. Available as "revised ethylbenzene sponsor document" at: http://www.tera.org/peer/VCCEP/Ethylbenzene/EBWelcome.html

### Appendix 1 Estimation of cumene animal and human systemic dose

Human dose:

1 ppm cumene = 4,916  $\mu$ g/m<sup>3</sup> (worker exposures are generally < 1ppm in cumene production andmanufacturing uses)

4,916  $\mu$ g/m<sup>3</sup> x 10 m<sup>3</sup> inhaled air per work shift = 49,160  $\mu$ g exposed per worker per work shift

49,160 μg x 0.5 (respiratory retention of cumene) = 24, 580 μg absorbed per 70 kg worker per day

24, 580 µg absorbed per 70 kg worker per day = 351 ug/kg/day = 0.35 mg/kg/day

Mouse dose at 1000 ppm:

1000 ppm = 4,916,180 μg/m<sup>3</sup>

4,916,180  $\mu$ g/m<sup>3</sup> x 0.0195 m<sup>3</sup> (respiratory volume inhaled in B6C3F1 mouse over a 6 hour inhalation

exposure; Chang et al., 1981) = 95,865  $\mu$ g cumene inhaled per 6 hr exposure

95,865  $\mu$ g cumene inhaled per 6 hr exposure x 0.5 (respiratory retention in mice) = 47,932  $\mu$ g absorbed per 6 hour exposure

47,932  $\mu$ g ÷ 0.026 kg bw mouse = 1,843,558  $\mu$ g/kg = 1,843 mg/kg/day systemic dose to mouse exposed to 1000 ppm.