Draft Report on Carcinogens Monograph for Cumene

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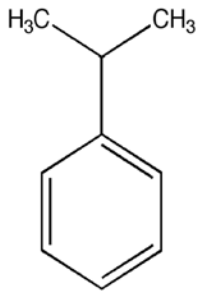
Properties and Use



- Cumene is a volatile liquid with a gasoline-like odor.
  - Component of fossil fuels: crude oil, coal tar, gasoline, and solvents. Found in cigarette smoke.
  - High production volume chemical, primarily used in the synthesis of acetone and phenol.
- Selected as a candidate substance for the RoC
  - Widespread current and past U.S. exposure.
  - An adequate database of studies in animals for evaluation of its potential carcinogenicity.

Exposure: Key Questions

- Is there significant exposure of the candidate substance to persons living in the United States?

- How are people (sources, settings, and levels) exposed to the candidate substance?

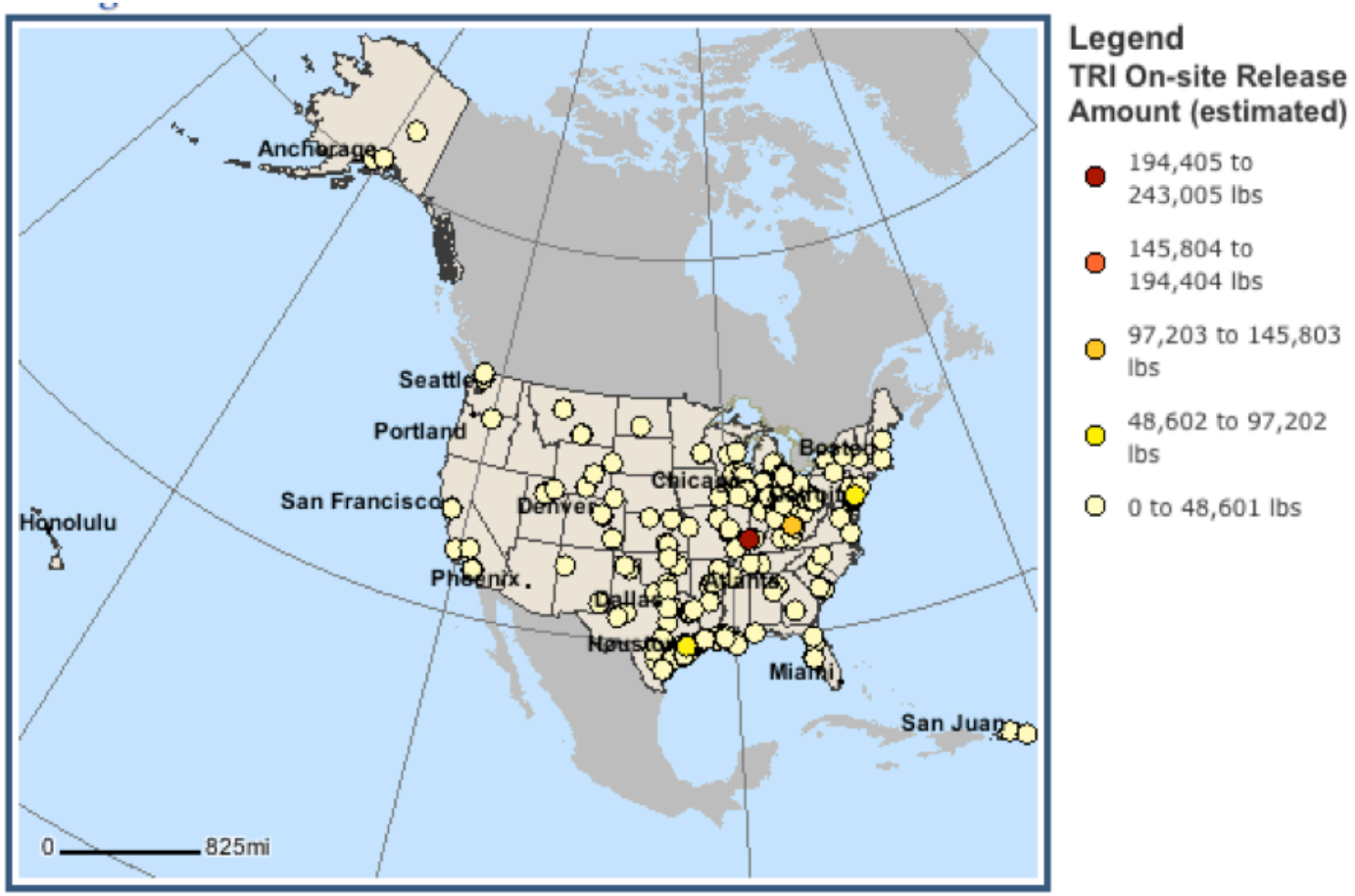
U.S. Exposure

- Occupational Exposures – production processes such as chemical syntheses, petroleum refining, rubber vulcanization, solvent and paint manufacture, and in pharmaceutical and textile industries.
  - Primary routes of exposure – inhalation or dermal exposures in workplaces.
  - Some of the highest levels of exposure during painting and car repair work.
- High U.S. Production
  - > 1 billion pounds per year
  - 2.29 billion pounds imported; 127 million pounds exported (2011)

U.S. Exposure

- Environmental Exposures
  - Contaminated air from combustion (e.g., motor exhaust) and evaporation (*e.g.*, blended gasoline and kerosene fumes) of fossil fuels; emissions from production, use, and transport, and from accidental chemical spills.
  - Primary route of exposure: inhalation of ambient air
  - Greater exposure in urban and industrial areas
  - Tobacco smoking
  - Trace levels in some foods
  - The amount of cumene released from gasoline distribution and use (23,509 kg/d) is greater than the release from production and use (17,903 kg/d).

Toxic Release Inventory Data: >1 million pounds from 300 facilities



Exposure

- Environmental exposure in Humans
  - Trace levels of cumene detected in expired air from non-smoking volunteers with no intentional exposure to cumene (Krotoszynski *et al.* 1977 and Conkle *et al.* 1975).
  - Cumene measured in blood hospital and chemical workers who were exposed to cumene from environment and not occupational duties. Blood levels were 40 times greater than expired air (Brugnone *et al*. 1989).

Exposure: Conclusions and Summary

There are a significant number of people residing in the U.S. that are exposed based on environmental and occupational data.

- Widespread environmental exposure from contaminated air from combustion (e.g., motor exhaust) and evaporation (*e.g.*, blended gasoline and kerosene fumes) of fossil fuels; emissions from production, use, and transport, tobacco smoke.
- Greater exposure in urban and industrial areas.
- High production volume chemical.
- Occupational exposure and release into the environment from manufacturing processes, painting, car repair work.

Cumene
Properties and Human Exposure

Questions or Clarifications?

Cumene
Properties and Human Exposure

- Comment on whether the chemical identity and description of cumene (Section 1: Properties and Human Exposure) are clear and technically accurate.

- Comment on whether the information on use, production, and human exposure for cumene (Section 1: Properties and Human Exposure and Appendix B) is clear and technically accurate.
  - Identify any information that should be added or deleted.

- Comment on whether adequate information is presented to document past and/or current human exposure to cumene in the United States. Exposure can be inferred by data on usage, production, or evidence for exposure in the workplace, from the environment or consumer products, diet, or other sources due to lifestyle choices (such as tobacco smoking).

Key Questions- Disposition and Toxicokinetics

- What is the scope of the database for cumene?

- Are there studies and information in humans and how do the results compare with what is known from animal studies?

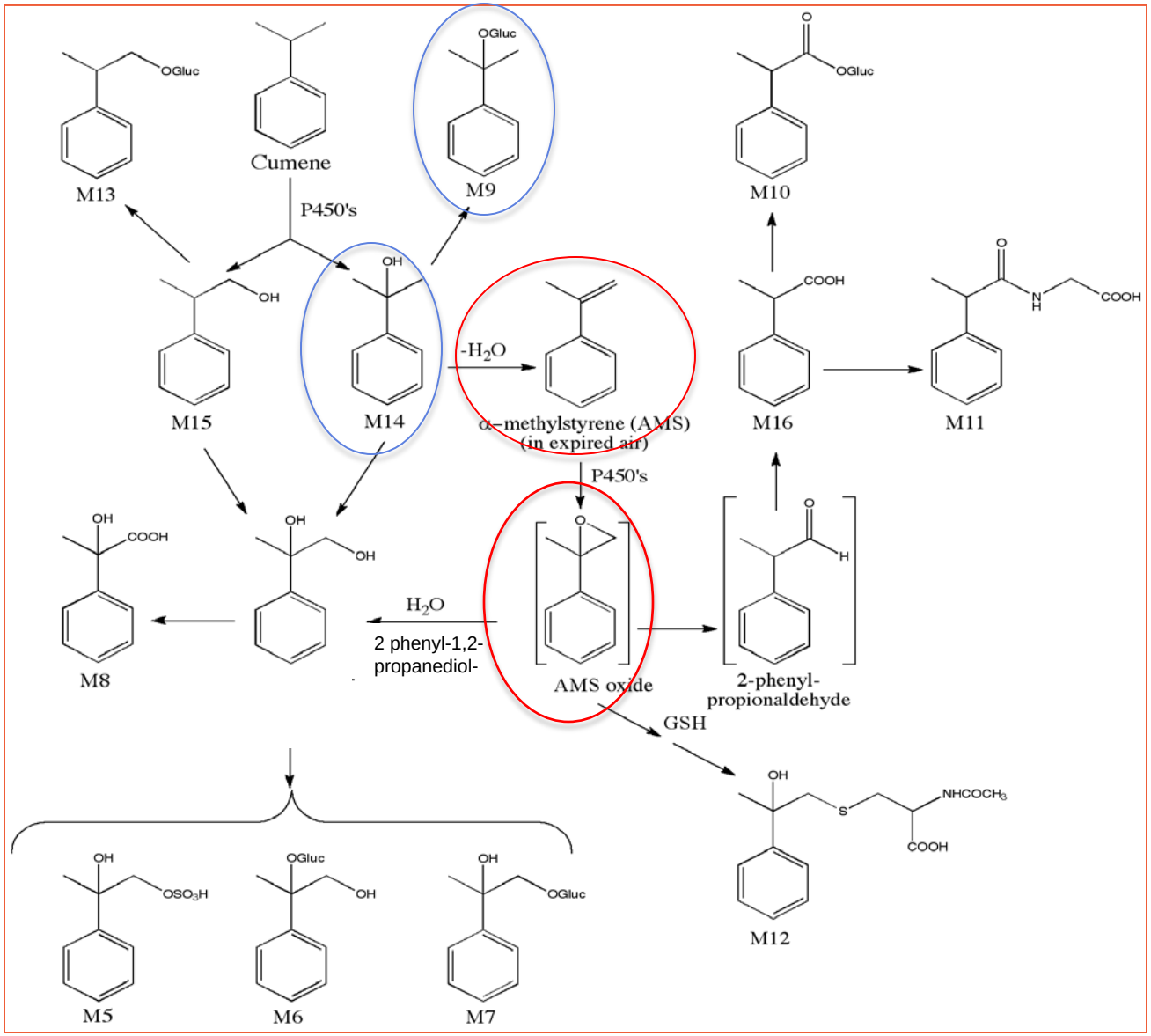
Disposition and Toxicokinetics

- Scope of the database for cumene
  - Most informative study by Chen *et al. (*2011), ADME study in rats (m) and mice (m,f) (oral and i.v.).
  - Studies in rats and rabbits (oral, dermal) (Robinson *et al.* 1955, Bakke and Scheline 1970, Ishida and Matsumoto, 1992).
  - One *in vitro* study investigated metabolism by cytochromes P450 (rabbit Cyp 4B1 and rat Cyp 2B1) using several substrates, including cumene (Henne *et al.* 2001). No other studies on cumene and cytochromes P450 were located.
- Studies in humans
  - One absorption and excretion study in humans (Senczuk and Litewka 1976).

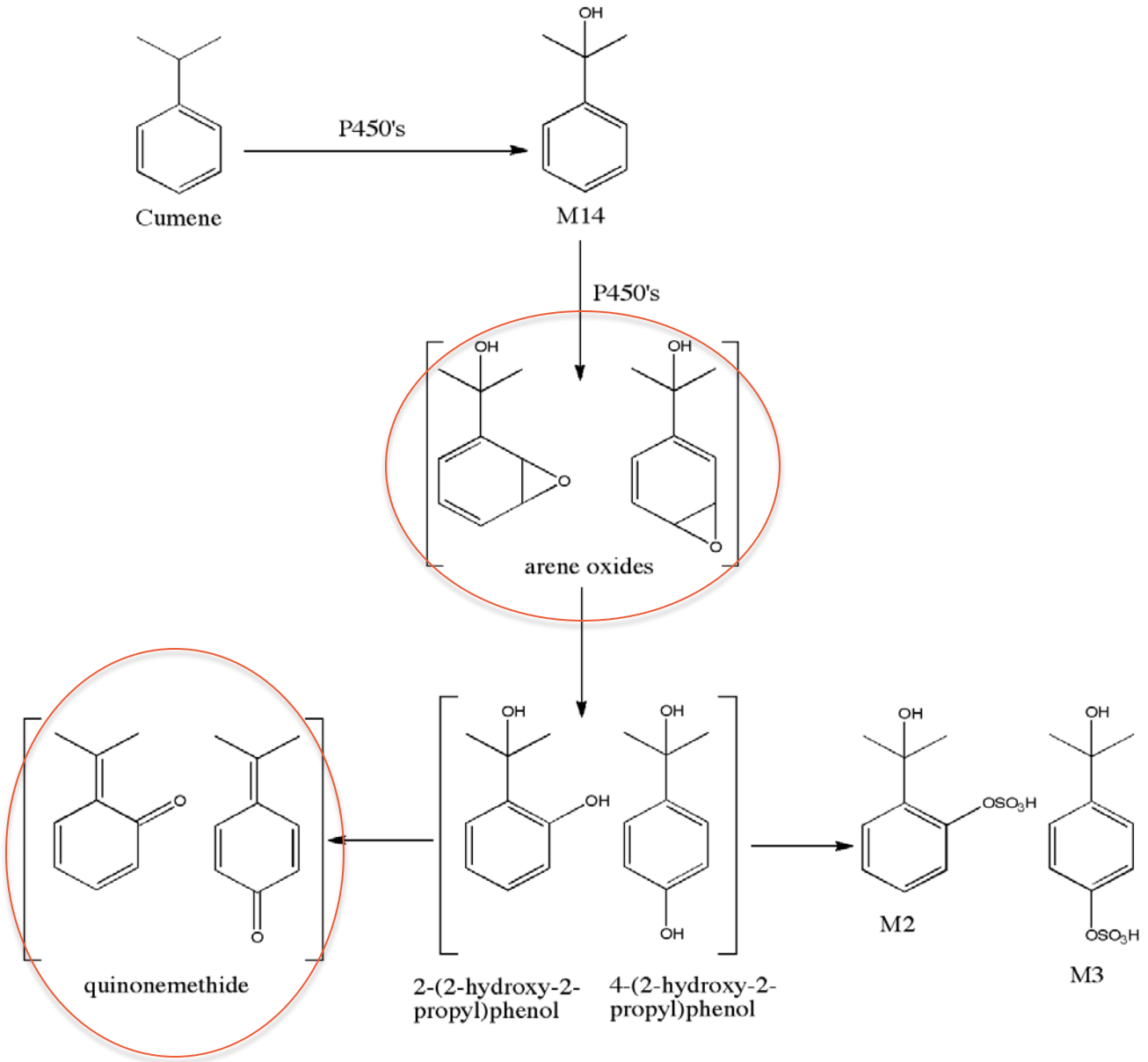
Disposition and Toxicokinetics

- Disposition and Metabolism (Chen *et al.* 2011)
  - Mice (m, f), rats (m) oral and intravenous dosing [^{14}C] cumene
    - Total of 16 metabolite peaks identified by HPLC radiochromatogram
    - P450 oxidation of alkane group (side-chain) or benzene ring
    - Primary metabolites are from side-chain oxidation
  - Microsomal incubations mouse (f) and rat (f) lung and liver
    - α–Methylstyrene and 2-phenyl-2-propanol produced in all cases

Metabolism: Side-Chain Oxidation Chen *et al.* 2011



Metabolism: Ring Oxidation Chen *et al.* 2011



Disposition and Toxicokinetics

- Studies in humans (Senczuk and Litewka, 1976)
  - Inhalation studies on human volunteers
  - Cumene absorption by inhalation was directly proportional to concentration of primary urinary metabolite, 2-phenyl-2-propanol.
- Metabolism by cytochrome P450
  - One *in vitro* study: rat CYP 2B1 formed 2-phenyl-2-propanol and rabbit CYP 4B1 formed 2-phenyl-1-propanol (Henne *et al.* 2001).

Disposition and Toxicokinetics: Summary

- Cumene is excreted in the urine primarily as 2-phenyl-2-propanol glucuronide in rats, mice, and humans.
- Cumene can undergo oxidation of the benzene ring or the alkyl side-chain potentially forming reactive intermediates.
- Side-chain oxidation can form alpha-methylstyrene
  - Identified in expired air and microsomal incubations
  - alpha-methylstyrene oxide is proposed reactive intermediate

Cumene
Disposition and Toxicokinetics

Questions or Clarifications?

Cumene
Disposition and Toxicokinetics

- Comment on whether the information on Disposition and Toxicokinetics (Section 2) is clear, technically correct, and objectively presented.
  - Identify any information that should be added or deleted.

Human Cancer Studies

No epidemiological studies were identified that examined the relationship between human cancer and exposure specifically to cumene.

Key Questions: Studies in Experimental Animals

- What is the level of evidence (sufficient or not sufficient) for the carcinogenicity of cumene from studies in experimental animals?
- What are the tissue sites?
- What is the scope of the literature?
  - One study met inclusion criteria as a cancer study.
  - NTP Technical Report (2009) – 2 yr. carcinogenesis studies of cumene in both sexes of F344/N rats and B6C3F_1 mice (inhalation studies)

Studies in Experimental Animals

Appendix C: study quality questions and assessment of animal cancer studies.

Study Quality Areas	Assessment
Substance Characterization	Purity >99.9%, stability monitored
Animal Husbandry	Animal source, care, housing, feed adequately described.
Study Design	Animal model, dose selection, route, duration, control animals described.
Clinical Observations, Necropsy, Pathology	Complete necropsies, tissue fixation method and tissue assessments described for neoplasia.
Data Reporting	Data presented in tabular format; statistical methods performed and described
Overall, is this study informative for a cancer assessment?	Yes, High quality study. There were no major limitations.

Studies in Experimental Animals

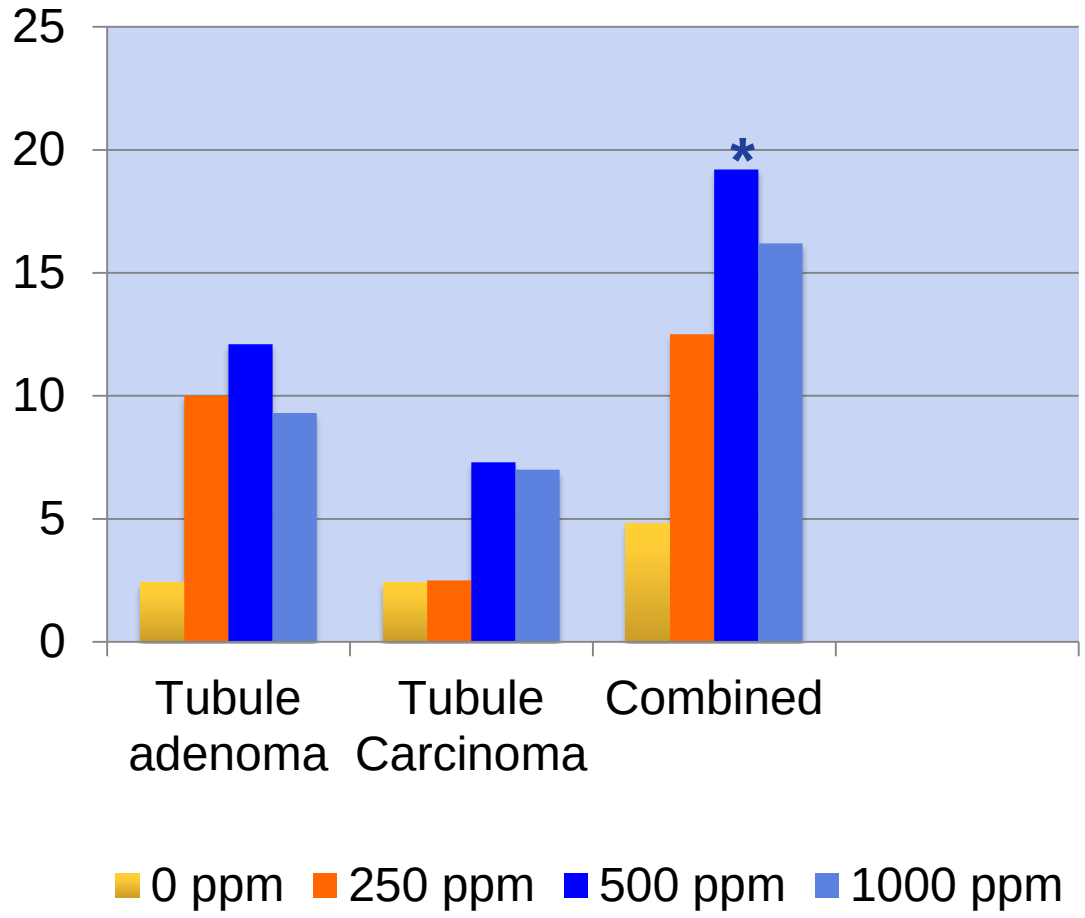
- NTP Technical Report (2009) – 2 yr. carcinogenesis studies of cumene in F344/N rats and B6C3F$_1$ mice (inhalation studies)
  - 6 hr/d for 5 d/wk
  - Dose setting for 2 yr. study based on results of subchronic study (14 wks)
  - Dose selection for 2 yr. study:
    - Rats (m, f) and Mice (m) 0, 250, 500, 1000 ppm
    - Mice (f) 0, 125, 250, 500 ppm
  - Renal toxicity study on subchronic core study rats
    - Right kidney: α_{2u}-globulin and soluble protein measured (m)
    - Left kidney: evaluation of hyaline droplets (m, f), cell proliferation indices (m), and histopathology (m, f)

Studies in Experimental Animals

NTP Technical Report (2009) chronic inhalation studies

Rat (F344/N)	Neoplastic Lesions
Male	Adenoma of respiratory epithelium of the nose Renal tubule adenoma and carcinoma (combined) Interstitial-cell adenoma of testes
Female	Adenoma of respiratory epithelium of the nose

Percent Incidence of Renal Tubule Neoplasia in Male F344/N Rats



* P≤ 0.05, compared with chamber controls

Historical controls: adenoma or carcinoma- 0-2%, combined- 0-4% for inhalation and by all routes

Hyperplasia of tubules: all cumene exposure groups; significance at 500 ppm of 8/50 (16%) P≤0.01; 1000 ppm 6/50 (12%) P≤0.05.

Studies in Experimental Animals

NTP Technical Report (2009) chronic inhalation studies

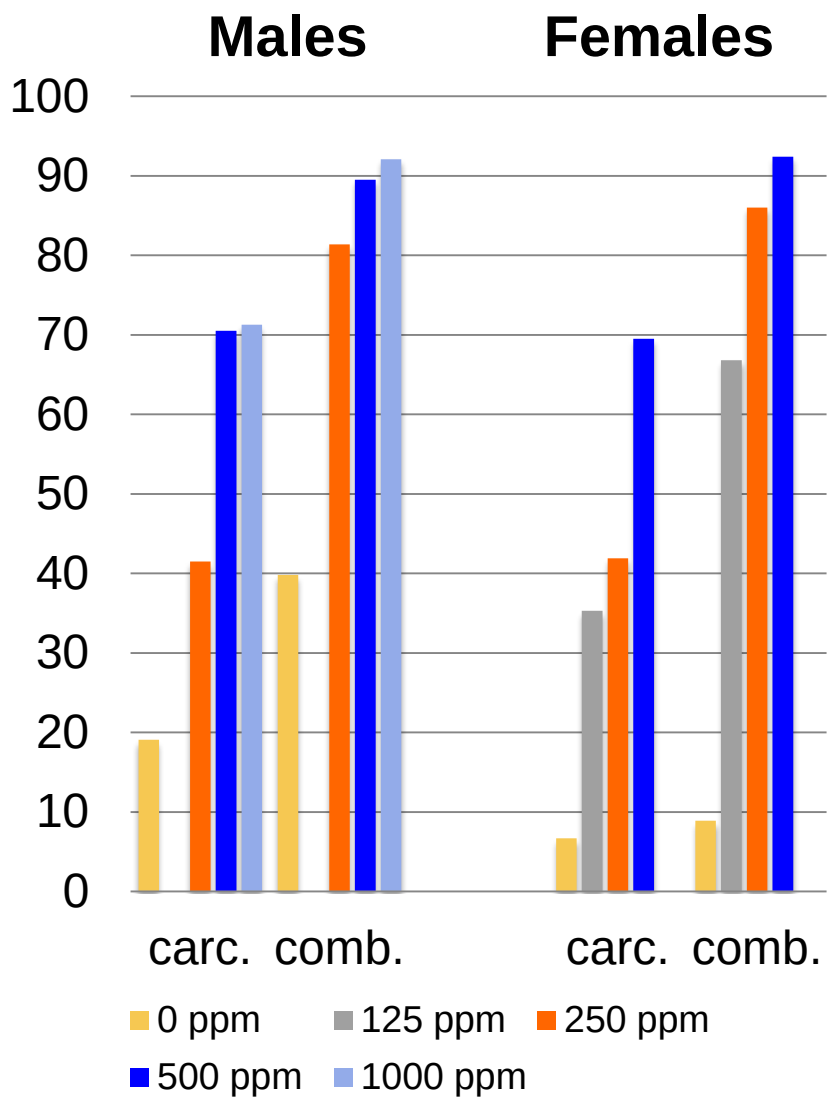
Rat (F344/N)	Neoplastic Lesions
Male	**Adenoma of respiratory epithelium of the nose** **-Typically do not progress** **Renal tubule adenoma and carcinoma (combined)** *Interstitial-cell adenoma of testes may have been exposure-related* **-Typically do not progress**
Female	**Adenoma of respiratory epithelium of the nose** **-Typically do not progress**

Studies in Experimental Animals

NTP Technical Report (2009) chronic inhalation studies

Mouse ($B6C3F_1$)	Neoplastic Lesions
Male	Alveolar/bronchiolar adenoma, carcinoma, or combined Hemangiosarcoma (spleen) Adenoma of the thyroid gland
Female	Alveolar/bronchiolar adenoma, carcinoma, or combined Hepatocellular adenoma or adenoma and carcinoma (combined)

Percent Incidence of Alveolar/Bronchiolar Tumors in B6C3F1 Mice

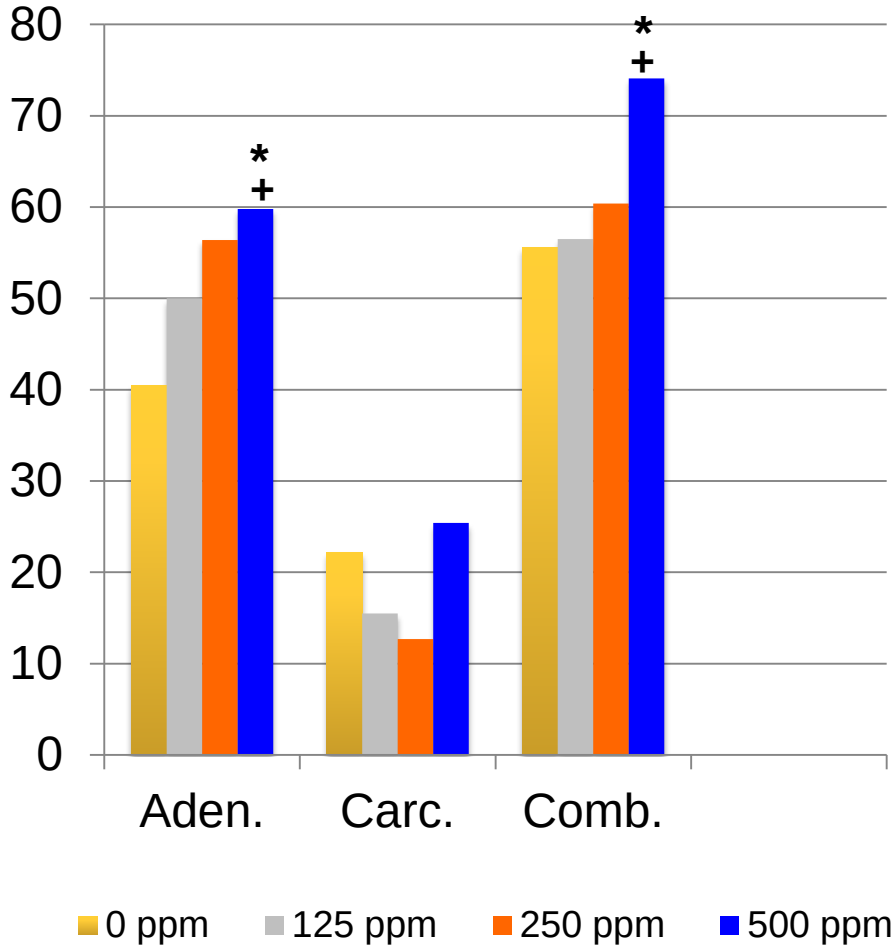


Poly-3 trend: $P < 0.001$ all tumor types & $P \leq 0.001$ pairwise for all exposure groups except male, carcinoma 250 ppm, $P \leq 0.05$

Preneoplastic lung lesions in males and females: bronchiolar hyperplasia and metaplasia of alveolar epithelium and bronchii significant at all cumene dose groups, $P < 0.01$

Historical controls (inhalation): males 10-24% carcinoma, 26-44% combined; females: 0-12% carcinoma, 2-14% combined

Percent Incidence of Hepatocellular Tumors in Female B6C3F1 Mice



+ Determined by poly-3 trend test, *P*= 0.040 adenoma, *P*= 0.024 combined

***** *P*≤ 0.05 compared with chamber controls

Historical controls
Adenoma: 12-36% inhalation, 2-62% all routes
Carcinoma: 6-20% inhalation, 0-28% all routes
Combined: 22-50% inhalation, 8-64% all routes

Eosinophilic foci: significant only in males, at 500 and 1000 ppm

Studies in Experimental Animals

NTP Technical Report (2009) chronic inhalation studies

Mouse ($B6C3F_1$)	Neoplastic Lesions
Male	**Alveolar/bronchiolar adenoma, carcinoma, or combined** *May have been exposure-related:* *Hemangiosarcoma (spleen)* **- within historical control range** *Adenoma of the thyroid gland* **- typically do not progress**
Female	**Alveolar/bronchiolar adenoma, carcinoma, or combined** **Hepatocellular adenoma or adenoma and carcinoma (combined)**

Studies in Experimental Animals
Preliminary Recommendation

There is sufficient evidence of carcinogenicity in experimental animals with an increased incidence of malignant and/or a combination of malignant and benign tumors in rats and mice or at multiple tissue sites.

- Combined benign and malignant **kidney tumors** in male rats.
- Benign, malignant, and combined **lung tumors** in male and female mice.
- Benign or combined with carcinoma **liver tumors** in female mice.

Studies in Experimental Animals

Questions or Clarifications?

Studies in Experimental Animals

- Comment on whether the scientific information from cancer studies in experimental animals for cumene (Section 4: Studies of Cancer in Experimental Animals and Appendix C) is clear, technically correct, and objectively presented.
- Comment on whether the assessment and integration of the scientific evidence (Section 4.2) are adequate to determine the level of evidence for carcinogenicity and to reach a listing recommendation.
  - Provide any scientific criticisms of the NTP's interpretation and application of the evidence from the cited studies in assessing the carcinogenicity of cumene.
  - Identify any information that should be added or deleted.

Key Questions: Mechanistic Data and Other Relevant Effects

- What are the potential modes of action by which cumene may cause cancer? Is there evidence that any mechanism is not relevant to humans?

- What is the level of evidence that the renal tumors observed in male rats are caused by an α_{2u}-globulin-associated renal nephropathy mechanism? Are there other potential mechanisms by which cumene could cause renal cancer in male rats?

Potential Modes of Action

- General: genetic or epigenetic effects?
- Mouse lung: formation of cytotoxic metabolites by CYP2F-specific mechanism?
- Kidney tumors in male rats: α_{2u}-globulin nephropathy?

Genotoxicity Studies

See Appendix D Sources: EC 2001, and WHO 1999. EPA 1997, Simmon 1997, Tardiff 1976; : Florin 1980, NTP 2009, NTP 2012; numbers of studies in parentheses.

In vitro

- Mutagenicity studies - bacteria (7), yeast (1), and mammalian cells (2)
  - Bacteria – negative; assay limitations: volatility of cumene, solvent system
  - Yeast and mammalian cells – negative; assay limitations: incomplete reporting of methods and results
- Chromosomal aberrations (1), cell transformation (2), unscheduled DNA synthesis (2)
  - Conflicted results; assay limitations: not reproducible, incomplete reporting of methods and/or high background

Genotoxicity Studies

In vivo

- Micronuclei
  - Mice (3) - Negative for micronuclei
  - Rats (2) - Conflicted results
- DNA damage (3)
  - Rats, FLARE assay (inadequate reporting, high background)
  - Rats, comet assay (positive, male liver)
  - Mice, comet assay (positive, female lung)
- Cumene was not mutagenic or genotoxic in most of the standard *in vitro* and *in vivo* assays. Cumene was positive for DNA damage in the comet assay.
- Comet assay can detect 90% of carcinogens that are negative or equivocal in micronucleus assay.

Mechanistic and Other Relevant Effects

Genotoxicity studies

- No data on DNA adducts for cumene or metabolites.
- Some evidence α-methylstyrene, a metabolite of cumene, is genotoxic.
  - Positive for micronuclei in mice
  - Positive for sister chromatid exchange in Chinese hamster ovary cells and in human lymphocytes.
- α-Methylstyrene oxide, an oxidation product of α-methyl-styrene, is mutagenic in the *S. typhimurium* assay.

Mechanistic and Other Relevant Effects

- Mouse lung tumors induced by cumene exposure positive for mutations in *K-ras* and *p53,* altered gene expression, loss of heterozygosity, and histone modifications.
  - These changes are different from what is found in spontaneous tumors (Hong *et al.* 2008).
    - Incidence of K-*ras* mutations in lung tumors: 87% cumene-induced, 14% spontaneous tumors.
    - Increase in G>T, A>G mutations in codons 12 and 61 K-*ras*
    - Mutation spectra and expression profiles similar to human cancers (Hoenerhoff *et al*. 2009).
  - Histone modifications in mouse lung tumors; associated with *K-ras* mutations (Wakamatsu *et al.* 2008).
    - Genes associated with histone deacetylase complex altered.
- Metabolism through side chain and ring oxidation to electrophilic intermediates could potentially cause DNA damage.

Mechanistic and Other Relevant Effects

- Hypothesis for pathogenesis of mouse **lung** tumors: Formation of cytotoxic metabolites by species-specific mechanism (cyp2f2)
  - *cyp2f2* mouse lung Clara cells, cytotoxic metabolites, regenerative hyperplasia leading to tumor formation.
  - Cytotoxicity studies, but no cancer studies on this MOA
  - Humans have lower levels of CYP2F1 in lung and fewer Clara cells in lung.
  - No available data on metabolism of cumene by *cyp2f2*.
  - No evidence of cytotoxicity in 3-month or 2-year studies.
  - No available data to discount human relevance.
- Data on mouse **liver** tumors are assumed relevant to humans.
  - No available data specific to cumene.
  - No available data to discount human relevance.

Mechanistic and Other Relevant Effects

Are **kidney** tumors in male rats caused exclusively by α_{2u}-globulin nephropathy, a species- and sex-specific mode of action?

- Convened a group of NTP scientists with specific expertise on nephropathy to independently evaluate data relevant to cumene exposure in adult male rats (90 day study and 2-year study) using IARC criteria and US EPA sequence of events for α_{2u}-globulin nephropathy.

IARC Criteria for α_{2u}-Globulin Nephropathy

CRITERIA	IARC CRITERIA MET ?
Nongenotoxic (agent or metabolite)	No, cumene weakly genotoxic; some evidence AMS is genotoxic
Male rat specificity for nephropathy and kidney tumors	Nephropathy (increased severity, incidence) also in females; Kidney weights increased males and females
Increase in hyaline droplet size and numbers in P2 segment	Yes
Identification of α_{2u}-globulin in tubule cells	Yes
Reversible binding to α_{2u}-globulin	No Data
Induction of sustained increased cell proliferation in the renal cortex	Weak evidence, no change labeling index
Dose response relationship of tumor outcome to histopathological endpoints (protein droplets, α_{2u}-globulin accumulation, cell proliferation)	Moderate to Strong Agreement

IARC Criteria for α_{2u}-Globulin Nephropathy

- "In making overall evaluation of carcinogenicity to humans, it can be concluded that production of renal-cell tumors in male rats by agents that fulfill all of the following criteria for an α_{2u}-globulin-associated response is not predictive of carcinogenic hazard to humans" (IARC 1999).

- Based on the IARC criteria and input from the NTP information group, we concluded that there is α_{2u}-globulin nephropathy, but could not exclude the possibility of an additional mechanism.

Cumene
Mechanistic Data and Other Relevant Effects

Questions or Clarifications?

Mechanistic Data and Other Relevant Effects

- Comment on whether the genotoxicity and other mechanistic data (Section 5: Mechanistic Data and Other Relevant Effects and Appendix D) presented in the cancer evaluation component for cumene are clear, technically correct, and objectively presented.
- Comment on whether the mechanistic data (Section 5: Mechanistic Data and Other Relevant Effects) are relevant for identifying and evaluating the potential mechanisms of action for the carcinogenic effects of cumene.
  - Provide any scientific criticisms of the NTP's interpretation and application of the genotoxicity data (Section 5.1: Genetic and related effects) from the cited studies for assessing effects of cumene.
  - Provide any scientific criticisms of the NTP's interpretation and application of the mechanistic data (Section 5.2: Mechanistic considerations) from the cited studies for assessing effects of cumene.
  - Identify any information that should be added or deleted.

Cumene: Overall Cancer Evaluation

- Sufficient evidence of cancer in experimental animals: Exposure resulted in benign and malignant tumors in two species of rodent at multiple tissue sites.
  - Lung tumors in male and female mice
  - Liver tumors in female mice
  - Kidney tumors in male rats
- No compelling evidence was identified to rule out the relevance of these tumors to humans.
  - Some evidence that cumene may cause DNA damage
  - Mouse lung tumor genotypes observed with exposure to cumene are similar to molecular alterations found in human lung and other cancers.

Cumene
Overall Cancer Evaluation

Preliminary listing recommendation:

Cumene is *reasonably anticipated to be a human carcinogen* based on sufficient evidence in experimental animals.

RoC Criteria: Page *iv* of the Draft RoC Monograph for Cumene

Cumene
Overall Cancer Evaluation

Questions or Clarifications?

Overall Cancer Evaluation

- Comment on the overall cancer evaluation (Section 6: Synthesis of Animal, Human, and Mechanistic Data) and NTP conclusion that “there are no compelling data to indicate that cumene causes cancer by mechanisms that would not occur in humans.”
  - Provide any scientific criticism of the NTP’s overall assessment and integration of the experimental animal and mechanistic data.

Draft Substance Profile

- Contains NTP's preliminary recommendation of the listing status of the substance.
- Summarizes the scientific information that is key to reaching a recommendation.
- Provides information on properties, use, production and exposure.
- Provides information on existing federal regulations and guidelines.

Cumene
Draft Substance Profile

Questions or Clarifications?

Draft Substance Profile

A. Comment on whether the information on use, production, and human exposure for cumene is clear and technically accurate.

B. Comment on whether the information presented regarding carcinogenicity and cancer studies in experimental animals is clear, technically correct, and objectively stated.

C. Comment on whether the substance profile highlights the key information from the cancer studies in experimental animal that supports the listing recommendation.

D. Comment on whether the information presented regarding studies on mechanisms of carcinogenicity and other relevant data is clear, technically correct, and objectively stated.

E. Comment on whether the substance profile highlights the studies on mechanisms of carcinogenicity and other relevant data that are key to providing support for evaluating the relevance of the cancer studies in experimental animals to human carcinogenicity.

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