Disposition and Metabolism of Cumene in F344 Rats and B6C3F1 Mice

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Nonstandard Abbreviations used in text:
  ADME, absorption, distribution, metabolism, and excretion; AMS, α-methylstyrene; BCA, bicinchoninic acid; BDC, bile duct-cannulated; CNS, central nervous system; ESI, electrospray ionization; MS/MS, tandem mass spectrometry; HPLC, high performance liquid chromatography; IV, intravenous; LD50, median lethal dose; LSC, liquid scintillation counter; MS, mass spectrometry; NMR, nuclear magnetic resonance; VOCs, volatile organic compounds
ABSTRACT

Cumene is a high-production-volume chemical that has been shown to be a central nervous system (CNS) depressant and has been implicated as a long-term exposure carcinogen in experimental animals. The absorption, distribution, metabolism, and excretion of \[^{14}\text{C}]\text{cumene} \,(\text{isopropylbenzene})\text{ was studied in male rats and mice of both sexes following oral or intravenous (IV) administration. In both species and sexes, urine accounted for the majority of the excretion (typically } \geq 70\%\text{) by oral and IV administration. Enterohepatic circulation of cumene and/or its metabolites was indicated as 37\% of the total dose was excreted in bile in BDC rats with little excreted in normal rats. The highest tissue }^{14}\text{C} \text{ levels in rats were observed in adipose tissue, liver, and kidney with no accumulation observed following repeat dosing up to 7 days. In contrast, mice contained the highest concentrations of }^{14}\text{C} \text{ 24 h post-dosing in the liver, kidney, and lung, with repeat dosing accumulation of }^{14}\text{C} \text{ observed in these tissues as well as in the blood, brain, heart, muscle, and spleen. The metabolites in the expired air, urine, bile, and microsomes were characterized with 16 metabolites identified. The volatile organics in the expired air were comprised mainly of cumene and up to 4\% }\alpha\text{-methylstyrene. The major urinary and biliary metabolite was 2-phenyl-2-propanol glucuronide, which corresponded with the main microsomal metabolite being 2-phenyl-2-propanol.}
INTRODUCTION

Cumene (isopropylbenzene) (Figure 1) is a natural constituent of crude oil, petroleum products, finished fuels, and is used as a thinner for paints, lacquers, and enamels (Merck, 1989; HSDB, 2005). It is also a high-production-volume chemical used in the manufacture of several other chemicals. Cumene is released into the environment during petroleum refining, burning, and evaporation and it is a constituent of cigarette smoke (Johnstone et al., 1962). It is estimated that 9500 tonnes of cumene are released into the environment annually (US EPA, 1988). Subsequently, there is human exposure to cumene in many occupational and environmental settings. Like other alkylbenzenes, cumene exhibits acute toxicity in animals as a CNS depressant. (Tegeris and Balster, 1994). In humans, cumene was readily absorbed following head-only inhalation exposure (Seńczuk and Litewka, 1976). Short-term exposure in humans has been reported to cause dizziness, drowsiness, slight incoordination, unconsciousness, and irritation to the eyes, skin, and respiratory tract (HSDB, 2005).

Acute exposure of mice to cumene vapor (2000 to 8000 ppm, 20 min) produced effects similar to those of CNS depressant drugs (Tegeris and Balster, 1994). Cumene was negative in in vivo and in vitro mutagenicity tests (except in a micronucleus assay in Fisher 344 rats, where cumene was weakly positive at a dosage that was lethal in some of the animals (HSDB, 2005; US EPA, 1997). However, when exposed to cumene vapor, higher incidence of alveolar/bronchiolar adenoma and carcinoma were observed in the lungs of mice of both sexes, but not in rats (NTP, 2009). Similar results were found for courmin (NTP, 1993), naphthalene (NTP, 1992; 2000), styrene (Cruzan et al., 1998, 2001), ethylbenzene (NTP, 1999), α-methylstyrene (AMS) (NTP, 2007a), divinylbenzene (NTP, 2007b), and benzofuran (NTP, 1989), which are all structurally related. In contrast, a higher incidence of adenoma and
carcinoma was observed in the kidney and not in the lung of male rats (and not female rats) versus controls in the 2-year toxicology and carcinogenesis studies conducted by the US National Toxicology Program (NTP, 2009). The LC$_{50}$ is 2000 ppm in mice that were exposed to cumene vapor for 7 h, and this exposure has been shown to cause varying degrees of toxicity to the liver, spleen, and kidneys (Werner et al., 1944). Increases in organ weights are the most prominent effects in both male and female rats exposed to 500 or 1200 ppm of cumene vapor 6 h per day, 5 days per week for 13 weeks (Cushman et al., 1995).

The oral LD$_{50}$ of cumene in rats and mice is 1400 mg/kg and 12750 mg/kg, respectively (Wolf et al., 1956; CDC, 1996). An increase in kidney weight was observed in female rats exposed to daily 462 or 769 mg/kg of cumene 5 days per week for 6 months by the oral route (Wolf et al., 1956).

Previous metabolism studies in rats of cumene have found 2-phenyl-2-propanol (M14) to be the major metabolite. Administration of radiolabeled cumene to F344 rats via IV injection, inhalation, or oral gavage demonstrated that greater than 70% of the doses were excreted in urine regardless of the route of administration, and M14 and its glucuronide or sulfate conjugates accounted for >50% of urinary excretion (US EPA, 1997).

To the best of our knowledge, there are no reported ADME studies of cumene in mice. Also, species and/or sex differences in the fate of cumene may account for the differential carcinogenic response observed in rats and mice in the 2-year NTP toxicity studies of cumene. The present studies were designed to investigate the ADME of single or consecutive daily doses of [$^{14}$C] cumene administered orally to male F344 rats or male and female B6C3F1 mice at doses ranging from 1.4 mg/kg to 1000 mg/kg (Table 1). The doses were based on the oral LD$_{50}$ of cumene, which was approximately 10-fold higher in mice than rats. The species and sex of
Experimental animals chosen were based on the potential carcinogenicity “clear evidence” designation from the long-term 2-year toxicology and carcinogenesis study by the National Toxicology Program (NTP, 2009). Animals also received [14C]cumene by IV injection to investigate the effects of dosing route on cumene disposition and to quantitate biliary excretion of cumene-derived radioactivity. Because the lung has been shown to be a specific target of cumene-mediated toxicity in the mouse and female mice were more susceptible than males (NTP, 2009), the metabolism of cumene was investigated in vitro using microsomes prepared from female mouse lung and liver tissues. Metabolism in female rat lung and liver microsomes were also studied for comparison.
METHODS

Materials

[Ring-U-\(^{14}\text{C}\)]-cumene (specific activity 54 mCi/mmol) was obtained from Moravek Biochemicals, Inc. (Brea, CA). \(^{14}\text{C}\)Cumene was supplied as a solution in ethanol (1 mCi/mL). The radiochemical purity was 98% as determined by high performance liquid chromatography (HPLC) analysis. Unlabeled cumene (purity 99%) was purchased from Acros organics (Fairlawn, NJ). Alkamuls-EL 620 (>99% castor oil ethoxylates) was obtained from Rhodia (Cranbury, NJ). Carbo-sorb E, Permafluor E\(^+\), and Ultima Gold scintillation cocktail were purchased from PerkinElmer (Boston, MA). In-Flow ES scintillation cocktail was obtained from IN\US Systems (Tampa, FL). Hydrogen peroxide (H\(_2\)O\(_2\), 30%) was purchased from Fisher Scientific (Pittsburg, PA). 2-Phenyl-2-propanol was purchased from Fluka (Switzerland). 2-phenyl-1-propanol, 2-phenyl-1,2-propanol; 2-hydroxy-2-phenylpropionic acid, 2-phenylpropionic acid, 2-phenylpropionaldehyde, AMS, thionyl chloride, glycine, trifluoroacetic acid, dithiothreitol, heparin, \(\beta\)-glucuronidase from Escheria coli (type VIIA, sulfatase-free), \(\beta\)-glucuronidase/sulfatase from Helix pomatia (type H-1), glucose 6-phosphate, glucose-6-phosphate dehydrogenase, and NADP\(^+\) were obtained from Sigma-Aldrich Co. (St. Louis, MO). AMS oxide was purchased from TCI America (Portland, OR). The bicinchoninic acid (BCA) Protein Assay Kit and albumin standard were purchased from Pierce Chemical Company (Rockford, IL).

Instruments

HPLC analyses were carried out on an Agilent (Santa Clara, CA) 1100 HPLC and an IN\US Systems \(\beta\)-RAM Model 3 radioactivity detector equipped with a lithium glass solid cell.
(500 µL) or a liquid cell (500 µL). When the liquid cell was used, In-Flow ES scintillation cocktail (IN\US Systems) was delivered in a 3:1 scintillation/elution ratio. A Luna C18, 5 µm, 4.6 x 150 mm column (Phenomenex, Inc., Torrance, CA) was used for disposition studies. The mobile phase included H2O (Solvent A) and acetonitrile (Solvent B). The elution began with a linear gradient from 10% B to 95% B over 15 min and held at 95% B for 5 min at a flow rate of 1.0 mL/min. The column compartment was maintained at 40°C and UV detection was at 254 nm. The retention time of cumene was 14.6 min.

A Varian Inc. (Walnut Creek, CA) Inertsil C8, 5 µm, 4.6 x 250-mm column was used for metabolism studies. The mobile phase included 0.1% trifluoroacetic acid in water (Solvent A) and 0.1% trifluoroacetic acid in acetonitrile (Solvent B). The elution began with a linear gradient from 0% B to 40% B over 30 min, then a linear gradient to 95% B over 10 min, and a linear gradient back to 0% B over 5 min at a flow rate of 1.5 mL/min. The temperature of the column compartment was not maintained and the UV detection was set at 254 or 210 nm.

ESI-MS and ESI-MS/MS were obtained on a PE Sciex API 365 Triple Quad Mass Spectrometer (Applied Biosystems, Foster City, CA). Samples were dissolved in methanol:water (1:1) and introduced to the mass spectrometer through direct infusion (50 µL/min) for either negative ionization (ESI(-)-MS or ESI(-)-MS/MS), or positive ionization (ESI(+)-MS or ESI(+)-MS/MS) analysis.

1H NMR spectra were acquired on a Bruker Avance 500 MHz nuclear magnetic resonance (NMR) spectrometer (Billerica, MA). The chemical shifts are reported in parts per million relative to D2O (4.8 ppm).
Animals

Male F344 rats (177–214 g, 9 weeks old), male B6C3F1 mice (25.2–28.9 g, 9 weeks old), and female B6C3F1 mice (17.1–22.2 g, 9 weeks old) were obtained from Charles River Laboratories (Wilmington, MA). Male bile duct-cannulated (BDC) F344 rats (248–275 g, 9 weeks old) were purchased from Hilltop Labs, Inc. (Scottdale, PA). Animals were housed individually in all-glass metabolism cages from 1 day prior to dosing until sacrifice and provided with food (Teklad Certified Rodent Diet (W) 8728C; Harlan Teklad, Madison, WI) and municipal water ad libitum. For microsomal preparations, animals were housed in shoebox cages before sacrifice. Animal studies were approved by the Lovelace Respiratory Research Institute Institutional Animal Care and Use Committee, conducted in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and carried out in compliance with the Guide for the Care and Use of Laboratory Animals (NRC, 1996).

Oral Dosing

Single and repeat doses were delivered by gavage. All oral doses were in corn oil, administered at 5 mL/kg to rats and 10 mL/kg to mice. The ratio (mg) of unlabeled cumene to \[^{14}\text{C} \text{cumene} \] was 34:1 and 2808:1 for rats and mice, respectively. The target doses administered by gavage to four male rats/treatment group were 1.4, 14, or 140 mg/kg. The means ± S.D. for the concentrations and amounts of \(^{14}\text{C} \) administered to rats were 1.7 ± 0.3 mg (92 ± 14 µCi)/kg, 18 ± 1 mg (90 ± 4 µCi)/kg, or 149 ± 10 mg (102 ± 7 µCi)/kg. The target doses administered to mice (n = 4) by gavage were 10, 50, 100, or 1000 mg/kg for male and 10, 150, or 1000 mg/kg for female. The means ± S.D. for the concentrations and amounts of \(^{14}\text{C} \) administered orally to mice
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were: 13 ± 0 mg (1129 ± 20 µCi)/kg, 53 ± 1 mg (1191 ± 0 µCi)/kg, 99 ± 1 mg (1127 ± 6 µCi)/kg, or 1071 ± 46 mg (1265 ± 54 µCi)/kg to males and 11 ± 0 mg (1129 ± 6 µCi)/kg, 151 ± 1 mg (1133 ± 7 µCi)/kg, and 1064 ± 59 mg (1504 ± 84 µCi)/kg to females.

IV Dosing

Single doses were delivered by IV injection into the tail vein of rats and mice. The ratio (mg) of unlabeled cumene to $^{14}$C cumene was 51:1 and 111:1 for rats and mice, respectively. The IV dose administered to rats and mice was in water/ethanol/Alkamuls (8:1:1 v/v/v) and administered at 1 mL/kg for rats and 4 mL/kg for mice. The target dose administered by the IV route to 3–4 male rats/treatment group was 1.4 mg/kg. The means ± S.D. for the concentrations and amounts of $^{14}$C administered to rats were 1.1 ± 0.0 mg (55 ± 2 µCi)/kg and 2.1 ± 0.1 mg (43 ± 2 µCi)/kg to noncannulated and BDC rats. The target dose administered by the IV route to 3 mice/treatment group was 10 mg/kg. The means ± S.D. for the concentrations and amounts of $^{14}$C administered to mice were 7 ± 0 mg (5 ± 0 µCi)/kg for males and 6 ± 2 mg (4 ± 1 µCi)/kg for females.

Sample collection and analysis

For single-dose studies, urine was collected and chilled by dry ice at 6, 12, and 24 h following administration for all studies, and at 48 and 72 h for the study lasting 72 h. For repeat-dose studies, urine was collected at 24-h intervals following administration. Urine from the urinary bladder collected from the euthanized animals was added to the last urine collection. At the end of each collection interval, the metabolism cages were rinsed with water or ethanol (after the terminal collection only) to calculate complete recovery of $^{14}$C excreted in urine. Bile was
collected from the BDC rats at 0.25, 1, 2, 3, 4, 5, 6, 12, and 24 h. Triplicate aliquots of urine, bile, and cage rinse were mixed with Ultima Gold scintillation cocktail and counted for $^{14}$C content in a PerkinElmer Model 2500 TR liquid scintillation counter (LSC). The remaining samples were stored at -20°C.

For single-dose studies, feces were collected, following administration, at 12 and 24 h for all studies and at 48 and 72 h for the study lasting 72 h. For repeat-dose studies, feces were collected at 24 h intervals. Fecal samples were homogenized with an approximately equal mass of water. The weight of the fecal homogenate was determined and triplicate aliquots were combusted in a Packard 307 Biological Sample Oxidizer, using Carbo-sorb E for trapping $^{14}$CO$_2$ and Permafluor E+ as the scintillation cocktail. The samples were then counted in the LSC for determination of $^{14}$C content.

Cumene-derived radioactivity expired as volatile organic compounds (VOCs) and CO$_2$ were collected by passing the air (flow = 200–600 mL/min) from the metabolism cage through a series of traps. VOCs and CO$_2$ were not collected in the repeat-dose studies and CO$_2$ was collected only in selected single-dose treatment groups. The first two traps contained ethanol or isopropanol (60 mL each) to collect VOCs and the following two traps contained 1 N NaOH (350 mL each) to collect CO$_2$. The first ethanol trap was chilled with wet ice and the second trap with a dry ice isopropanol slurry to inhibit evaporation of the solvents. The isopropanol and NaOH traps were chilled with wet ice. All traps were changed at 6 and 12 h post-dosing. Duplicate aliquots of trapping solution were weighed individually into separate scintillation vials containing Ultima Gold scintillation cocktail and analyzed for $^{14}$C content in the LSC.

Bile was collected from the BDC rats at 0.25, 1, 2, 3, 4, 5, 6, 12, and 24 h following administration of cumene. All samples were stored at -20°C before analysis.
Blood was collected into a heparinized syringe by cardiac puncture from anesthetized animals prior to sacrifice. Adipose tissue (perirenal and reproductive), muscle (hind leg and trapezius), and skin (ears) were collected. In order to estimate the total contribution to those tissues their total content was estimated as 7.0, 40.4, and 19.0% of the body weight for rats and 7.0, 38.4, and 16.5% for mice. The following entire organs were collected and weighed: brain, lung, heart, spleen, kidneys, urinary bladder, uterus, liver, thyroid, stomach with contents, small intestine with contents, cecum with contents, and the remaining large intestine with contents.

Blood, adipose, muscle, skin, brain, lung, heart, spleen, kidney, testes/uterus, urinary bladder, thyroid, and aliquots of homogenized liver were combusted and counted as described above for fecal homogenates. The stomach and small intestines with their contents, the cecum and the remaining large intestine with their contents, and the residual carcass were solubilized in 2N NaOH in ethanol. For the IV study, the tail was removed, solubilized in 2N NaOH in ethanol, and analyzed for residual $^{14}$C. Once dissolved, these samples were neutralized with nitric acid and bleached with $\text{H}_2\text{O}_2$ (30%). Three aliquots of these samples were weighed into scintillation vials containing Ultima Gold scintillation cocktail and analyzed for $^{14}$C in the LSC.

Anesthesia and euthanasia

At the end of all studies animals were administered a sodium pentobarbital-based solution by i.p. injection to induce surgical-level anesthesia and euthanized by exsanguination and sectioning of the diaphragm.
Metabolite Isolation

Metabolite isolation was carried out on excreta collected within 24 h of dosing. Metabolites were isolated from HPLC by collecting the radiolabeled peaks detected with a β-ram solid cell or by collecting the UV-absorbing peaks with the detection at 254 nm. The collected samples were placed under a stream of N₂ to remove acetonitrile and then lyophilized in a model 77510 Labconco FreeZone 4.5-liter freeze dry system (Kansas City, MO) to remove water, or the solvents were evaporated by a Speed-Vac® (Thermo-Savant, Waltham, MA).

β-Glucuronidase and/or sulfatase hydrolysis of urine and bile samples

Urine samples (30 µL) were incubated with β-glucuronidase from E. coli (Type VII, ~2000 U, sulfatase-free) or β-glucuronidase/sulfatase from H. pomatia (β-glucuronidase ~2000 U and sulfatase ~67 U) in a 0.1-M sodium acetate buffer (pH 6.8 for the enzyme from E. coli and pH 5.0 for the enzymes from H. pomatia). Controls were prepared by using enzymes that had been heat deactivated (boiled for 10 min). Total volumes were 160–210 µL. Incubations were maintained at 37°C overnight and then analyzed by HPLC.

Preparation of liver and lung microsomes

Liver and lung microsomes were prepared from four female F344 rats and 10 female B6C3F1 mice. All procedures were carried out at 0–4 °C. Livers were homogenized in 9 volumes of 0.25 M sucrose. Lungs were homogenized in 3 volumes of 10 mM Tris, 150 mM KCl, 1 mM EDTA, 0.5 mM dithiothreitol, and 15% glycerol (pH 7.4). After centrifugation of the homogenate at 9,000 g for 10 min, the supernatant was removed and centrifuged at 100,000 g for 1 h. The lung microsomal pellet was resuspended in 10 mM Tris, 150 mM KCl, 1 mM
EDTA, and 15% glycerol (pH 7.4) then aliquoted and stored at -80°C. The liver microsomal pellet was resuspended in 10 mM Tris, 1 mM EDTA, and 20% glycerol (pH 7.4) then aliquoted and stored at -80 °C.

**Incubations of cumene with microsomes and the NADPH regenerating system**

[^14C]Cumene (1 mM, 1 μCi per sample) was incubated with microsomes (1 mg protein/mL) in a 0.1-M potassium phosphate buffer (pH 7.4) in the presence of 3 mM MgCl2, 25 mM glucose 6-phosphate, glucose-6-phosphate dehydrogenase (2 units/mL), and 4 mM NADPH. Cumene was added as an acetonitrile solution (100 mM, 0.1 μCi/μL, 10 μL). Control experiments omitting NADPH were included. The final volume was 1 mL and the incubation took place at 37°C in capped vials for 30 min. The reactions were terminated and proteins precipitated by addition of 1-mL of ice-chilled acetonitrile. Following centrifugation (10,000 g, 5 min), the supernatant was analyzed by HPLC.

**Synthesis of 2-phenylpropionylglycine**

2-Phenylpropionic acid (258 μL, 283.3 mg, 1.83 mmol) and 1.5 eq. thionyl chloride (200 μL, 326.2 mg, 2.74 mmol) in dichloromethane (10 mL) was stirred in an ice bath for 1.5 h. The mixture turned yellow while bubbles evolved from the solution. Triethylamine (382 μL, 277.3 mg, 2.74 mmol) was added slowly to the resulting mixture that was on ice. A white precipitate was formed during the addition. After all of the triethylamine was added, glycine (138.8 mg, 1.83 mmol) was added and the mixture was stirred at room temperature overnight. The solvent was evaporated to dryness under a stream of N2 and the residue was dissolved in methanol:water (1:1) for HPLC analysis. A peak with a retention time at 22.0 min was collected. The solvents...
were evaporated by a Speed-Vac® (Thermo Savant, Waltham, MA) and the residue was analyzed by MS and $^1$H NMR. The mass spectra [ESI(-)-MS: m/z 206 [M - H]$^-$; ESI(-)-MS/MS of m/z 206: m/z 162 [M - H - CO$_2$]$^-$; ESI(+)-MS: m/z 208 [M + H]$^+$; ESI(+)-MS/MS of m/z 208: m/z 190 [M + H – H$_2$O]$^+$, 133 [M + H - glycine]$^+$, 105 [M + H - glycine - CO]$^+$] and NMR spectra [$^1$H NMR (D$_2$O): $\delta$ 7.46-7.39 (m, 5H, phenyl-H), 3.96 (AB quartet, J = 17.5 Hz, 2H, Gly $\alpha$-CH$_2$), 3.88 (q, J = 7.0 Hz, 1H, 2-CH), 1.53 (d, J = 7.0 Hz, 3H, 3-CH$_3$)] confirmed the identity of the 2-phenylpropionylglycine.

**Statistical Analysis**

Statistical analysis of data was carried out using GraphPad Prism f-test and two-tailed t-test.

Values were considered significantly different at $p \leq 0.05$. 
RESULTS

Excretion of cumene-derived radioactivity

The excretion data from all $[^{14}\text{C}]$cumene studies are shown in Table 2. Approximately 70–80% of the total $^{14}$C was excreted in urine and 1% excreted in feces 24 h after administration of single oral doses in the range of 1.4 to 140 mg/kg. Most of the nonexcreted dose was putatively located in the intestinal contents 24 h post-dosing. Little $^{14}$C remained in the intestines and excretion of dose was higher in urine 72 h following gavage administration of 14 mg/kg. Additionally, the amount of $^{14}$C contained in the intestines 24 h following the last of 3 or 7 consecutive daily doses of 14 mg/kg was significantly less compared to the amount observed in the intestines 24 h following single administration of the same dose. Excretion of radioactivity as $^{14}$CO$_2$ in all surveyed groups was negligible (data not shown). A small, but measurable quantity of $^{14}$C was excreted as VOCs following single-dose administration of cumene to rats. Recovery of $^{14}$C as VOCs from the 140 mg/kg oral dose was higher relative to the lower oral doses ($p = 0.03$). IV-injected rats excreted more $^{14}$C as VOCs than did rats gavaged with a similar dose. In BDC rats, 37 ± 14% of an IV dose of 1.4 mg/kg was excreted in bile within 24 h of cumene administration.

Mice (male and female) primarily excreted $^{14}$C in the urine following administration of $[^{14}\text{C}]$cumene. However, in contrast to rats, little $^{14}$C remained in the intestines of mice 24 h after dosing. There was a trend toward decreased excretion in urine with increasing dose; however, due to variance between individual animals the only statistically significant differences observed were between the low and high doses for both males and females ($p = 0.001$ and 0.005 for males and females, respectively). Male and female mice excreted significantly more $^{14}$C as VOCs at the high dose relative to the lower doses ($p = 0.005$ and 0.002 for males and females,
respectively). As in rats, excretion of $^{14}$CO$_2$ was negligible; however, female mice excreted less $^{14}$CO$_2$ (0.03 ± 0.01%) at the high dose than did males (1.6 ± 0.1%) ($p = 0.0001$). Females also excreted more $^{14}$C as VOCs at the high dose than did males ($p = 0.02$). Little or no sex-related differences in $^{14}$C excretion were observed in the range of middle doses (50–150 mg/kg) administered to mice. The pattern of $^{14}$C excretion following IV administration was similar to that following administration of a similar oral dose to mice. However, the amount of $^{14}$C excreted in urine was less following the IV dose, possibly due to poor recovery of dose, particularly in the male mice. Repeat oral dosing had little or no effect on the excretion of $^{14}$C following daily administration of 150 mg/kg for either three or seven consecutive days to female mice.

**Tissue distribution of cumene-derived radioactivity**

Across the range of administered single doses, tissues (excluding stomach and intestines) contained less than 1 and 3% of the total dose in mice and rats, respectively, 24 h post-dosing (data not shown). Generally, the highest concentrations of residual cumene-derived radioactivity detected in tissues of these animals were in the liver, kidney, and lung and these data, including blood, are presented in Table 3. The increases in the concentrations of $^{14}$C in the blood, liver, kidney, and lung were proportional to dose in the range of 1.4–140 mg/kg in the rat; however, the data were more variable across the range of doses in mice. The concentrations of $^{14}$C in these tissues were higher in the rat than in the mouse at comparable doses in the range of 10–150 mg/kg. In mice, concentrations of $^{14}$C were similar in respective tissues of males and females following gavage of 10 mg/kg; however, tissues of female mice generally contained higher
amounts of $^{14}$C than those of male mice following administration of 1000 mg/kg. The tissue to blood ratio for liver, kidney, and lung was greater than 1 in all single-dose treatment groups. Repeat-dose studies were conducted in male rats and female mice to investigate potential accumulation of cumene-derived radioactivity in tissues. The animals received three or seven daily middle doses (14 mg/kg and 150 mg/kg for rats and mice, respectively) by gavage and the tissue concentrations of $^{14}$C were determined 24 h after the last dose (Table 4). There were no significant differences in concentrations of $^{14}$C in liver, kidney, and lung in the rat following repeat dosing compared to those observed after a single dose. Concentrations of $^{14}$C in blood, muscle, skin, and spleen were significantly increased after three doses in the rat; however, only skin contained significantly elevated concentrations of $^{14}$C after seven doses. Cumene-derived radioactivity was higher in female mouse liver, kidney, lung, blood, brain, heart, muscle, and spleen after three or seven doses compared to a single dose. Concentrations of $^{14}$C in blood, heart, kidney, and lung increased between three and seven doses. As shown in Figure 2, kidney and liver contained more $^{14}$C than lung and blood after one, three, or seven doses in the rat. Excluding the rat urinary bladder, the kidney of the rat had the highest tissue to blood ratio of the tissues in all treatment groups as shown in Table 4. The high variance in concentrations in the $^{14}$C in the urinary bladder indicated that the $^{14}$C was primarily associated with residual urine, rather than the tissue itself. In the mouse, the lung and liver contained the highest mean concentrations of $^{14}$C among the blood, liver, kidney, and lung (Table 4 and Figure 2). The lung appeared to have the greatest potential among these tissues for accumulation of $^{14}$C over time.

**Expired Air Metabolism**
HPLC analysis of the expired VOCs (0–6 h) from male mice treated with a 1000 mg/kg oral dose showed cumene (retention time 40.6 min) and a radiolabeled peak eluting at 39.8 min (Figure 3A). This radiolabeled peak at 39.8 min had a strong UV absorption at 254 nm (Figure 3B). The metabolite at 39.8 min was suspected to be AMS due to the strong UV absorption at 254 nm and showed a similar polarity to that of cumene. HPLC analysis of authentic AMS demonstrated a similar retention time as that of the metabolite (data not shown). The integration of the HPLC-radiolabeled peaks in the expired air gave AMS:cumene ratios of 3:97 for male mice (Figure 3A and 3B), 4:96 for female mice treated with a 1000 mg/kg oral dose, and 0:100 for male rats treated with a 140 mg/kg oral dose, although the UV peak of AMS was observed in the expired VOCs of male rats. Other treatment groups excreted less 14C as VOCs, and therefore the VOCs was not analyzed.

**Urinary Metabolites**

Seventy percent and higher of cumene-derived radioactivity was excreted in urine of all treatment groups within 24 h of dosing. HPLC analysis (liquid cell) of the urine collected 24 h from male rats and mice of both sexes dosed with high oral doses revealed a number of radiolabeled peaks designated as M1–16 (Figure 4A–4C). Some of the urine samples were subject to hydrolysis by glucuronidase/sulfatase from *H. pomatia* or glucuronidase from *E. coli* (sulfatase-free) in order to recognize glucuronide or sulfate conjugates. A representative HPLC radiochromatogram from hydrolysis of male rat urine is shown in Figure 4D. Cumene was not detected in urine (data not shown). The urinary metabolites were characterized by MS and/or 1H NMR analysis (see supplemental info.). The structures of the identified metabolites are shown in Figure 5.
M1 with an HPLC retention time of 10.1 min was present in mouse urine but not in rat urine. This metabolite was not hydrolyzed by glucuronidase or sulfatase and did not ionize upon MS analysis. HPLC re-analysis of the purified metabolite showed not only the metabolite at 10.1 min but also another two radiolabeled peaks at 16.4 and 17.0 min, likely the dehydration products. The structure of M1 remains unknown, but the data suggest that it is likely a Phase I metabolite and may be a dihydrodiol, which can dehydrate to form two phenols.

M2 with an HPLC retention time of 13.4 min is more abundant in mouse urine than in rat urine (Figure 4). M2 from female and male mouse urine was hydrolyzed by sulfatase. ESI(-)-MS of M2 gave a molecular weight of 232, which is equivalent to cumene (120) + 2 [O] + SO₃ (80), a dihydroxycumene monosulfate. The NMR spectrum of M2 showed two CH₃ groups as a singlet at δ 1.55, consistent with hydroxylation at the 2-position of the isopropyl side chain. Because both CH₃ groups were intact, the second hydroxylation must have occurred at the phenyl ring. The coupling of the aromatic protons in M2 suggested the ring oxidation was at the ortho-position. HPLC re-analysis of the purified metabolite showed not only M2 at 13.4 min but also another radiolabeled peak at 28.7 min, with a strong UV absorption at 254 nm. This decomposed product was also observed by NMR, which showed resonances at δ 5.41 (s, 1H, olefin H), 5.11 (s, 1H, olefin H), and 2.14 (s, 3H, 3-CH₃), the other signals were not well resolved. The HPLC re-analysis and NMR were consistent with formation of an AMS derivative from dehydration of M2. This metabolite was identified as 2-(2-hydroxy-2-propyl)phenylsulfate (M2).

M3 with an HPLC retention time of 13.8 min was hydrolyzed by sulfatase, and the hydrolysis product had an HPLC retention time at 17.8 min (Figure 4D). ESI(-)-MS of M3 gave
a molecular weight of 232, equivalent to cumene (120) + 2 [O] + SO₃ (80), a dihydroxycumene monosulfate. The NMR spectrum of M₃ showed two CH₃ groups as a singlet at δ 1.62 and only four phenyl protons at δ 7.59 and δ 7.34 as two doublets, consistent with hydroxylation at the 2-position of the isopropyl side chain and the para-position of the phenyl ring. HPLC re-analysis of the purified metabolite showed not only M₃ at 13.8 min, but also another radiolabeled peak at 28.0 min with a strong UV absorption at 254 nm. This decomposed product also was observed by NMR, which showed resonances at δ 7.66 (d, J = 8.5 Hz, 2H, phenyl 3,5-H), 7.48 (d, J = 7.5 Hz, 2H, phenyl 2,6-H), 5.20 (s, 1H, olefin H), and 5.50 (s, 1H, olefin H), 2.20 (s, 3H, 3-CH₃). The HPLC re-analysis and NMR were consistent with formation of an AMS derivative from dehydration of M₃. The peak at 28.0 min was further hydrolyzed by sulfatase to give a hydrolysis product with an HPLC retention time at 35.0 min, suggesting that the sulfate conjugation was on the phenol group. This metabolite was identified as 4-(2-hydroxy-2-propyl)phenylsulfate (M₃).

M₄ with an HPLC retention time of 14.4 min was not hydrolyzed by glucuronidase or sulfatase (Figure 4D). This metabolite did not ionize upon ESI-MS analysis. HPLC re-analysis of the purified metabolite showed not only M₄ at 14.4 min, but also another two radiolabeled peaks at 22.6 and 23.1 min, likely the dehydration products. The structure of M₄ remains unknown, but the data suggest that it is likely a Phase I metabolite.

M₅ with an HPLC retention time of 15.2 min was hydrolyzed by sulfatase. The molecular weight (232) is consistent with a dihydroxycumene monosulfate (cumene (120) + 2 [O] + SO₃ (80)). MS fragmentation shows loss of one SO₃. When the metabolite was isolated by HPLC solid cell, subsequent HPLC re-analysis showed that it had decomposed to a peak with a similar retention time as that of 2-phenyl-1,2-propanediol (17.6 min). When the metabolite was
isolated by HPLC UV cell it was stable for a $^1$H NMR analysis. All five phenyl protons were observed by NMR, indicating that there was no ring oxidation. The presence of 3-CH$_3$ as a singlet and 1-CH$_2$ as an AB quartet indicated that hydroxylation occurred at the 1- and 2-positions of the isopropyl side chain. The NMR spectrum of authentic 2-phenyl-1,2-propanediol was as follows: $\delta$ 7.55 (d, $J = 7.5$ Hz, 2H, phenyl 2,6-H), 7.49 (t, $J = 7.5$ Hz, 2H, phenyl 3,5-H), 7.40 (t, $J = 7.5$ Hz, phenyl 4-H), 3.76 (AB quartet, $J = 12.0$ Hz, 2H, 1-CH$_2$), and 1.57 (s, 3H, 3-CH$_3$). Due to sulfate conjugation, the 1-CH$_2$ AB quartet in M$_5$ ($\delta$ 4.17) was more downfield compared to that in the hydrolysis product ($\delta$ 3.76). The position of sulfate conjugation was likely at the 1-position, which was less hindered. This metabolite was tentatively identified as 2-hydroxy-2-phenylpropylsulfate (M$_5$).

MS analysis of M$_6$ with an HPLC retention time of 16.6 min gave a molecular weight of 328, equivalent to cumene (120) + 2 [O] + glucuronide (176). The NMR spectrum of M$_6$ showed signals corresponding to glucuronide protons. All five phenyl protons were observed by NMR, indicating that there was no ring oxidation. The presence of 3-CH$_3$ as a singlet and 1-CH$_2$ as an AB quartet indicated that hydroxylation occurred at the 1- and 2-positions of the isopropyl side chain. The spectral data of M$_6$ were consistent with formation of a 2-phenyl-1,2-propanediol monoglucuronide. The position of glucuronide conjugation was likely at the 2-position, as the steric effect might have resulted in 3-CH$_3$ and 1-CH$_2$ being further downfield in M$_6$ than in its isomer, M$_7$. This metabolite was identified as 2-phenyl-1,2-propanediol 2-glucuronide (M$_6$).

M$_7$ with an HPLC retention time of 17.6 min had a molecular weight of 328, consistent with formation of a dihydroxycumene monoglucuronide (cumene (120) + 2 [O] + glucuronide (176)). The NMR spectrum of M$_7$ showed five phenyl protons, indicating no ring oxidation. The presence of 3-CH$_3$ as a singlet and 1-CH$_2$ as an AB quartet indicated hydroxylation at the 1-
and 2-positions of the isopropyl side chain. β-Glucuronidase hydrolysis of this glucuronide gave an aglycone with the same HPLC retention time (17.6 min) to that of the parent glucuronide and of the 2-phenyl-1,2-propanediol authentic standard. Epimerization of glucuronide 1’-H took place during isolation, so the signals attributed to the α-glucuronide were also observed by NMR (data not shown). All data suggested formation of 2-phenyl-1,2-propanediol monoglucuronide.

Because M7 was more abundant than its isomer M6, the position of glucuronide conjugation was likely at the less hindered 1-position. A previous metabolism study showed formation of 2-phenyl-1,2-propanediol 1-glucuronide as a major metabolite of AMS (De Costa et al., 2001).

M8 had a similar HPLC retention time (20.3 min) to that of authentic 2-hydroxy-2-phenylpropionic acid. MS analysis showed this metabolite had a molecular weight of 166, consistent with the molecular weight of 2-hydroxy-2-phenylpropionic acid. The NMR data of M8 are also identical with those of authentic 2-hydroxy-2-phenylpropionic acid. This metabolite was identified as 2-hydroxy-2-phenylpropionic acid (M8).

M9 with an HPLC retention time of 21.7 min had a molecular weight of 312, consistent with a monohydroxycumene glucuronide (cumene (120) + [O] + glucuronide (176)). MS/MS analysis showed loss of a glucuronide anion. 1H NMR analysis demonstrated signals corresponding to glucuronide protons and two three-proton singlets at 1.71 and 1.62 ppm (1-CH3 and 3-CH3), in agreement with hydroxylation of cumene at the 2-position of the isopropyl side chain followed by glucuronidation. Due to the presence of the chiral glucuronide, the two CH3 groups were not equivalent, and therefore had different chemical shifts. β-Glucuronidase hydrolysis of this metabolite gave an aglycone with an HPLC retention time at 27.6 min, identical to that of 2-phenyl-2-propanol. This metabolite was identified as 2-phenyl-2-propanol glucuronide (M9).
M10 co-eluted with M9 at 21.7 min. MS analysis of the peak at 21.7 min from rat urine indicated that M10 had a molecular weight of 326, consistent with formation of 2-phenylpropionylglucuronide (2-phenylpropionyl acid (150) + glucuronide (176)). β-glucuronidase hydrolysis of the peak at 21.7 min from rat urine gave 2-phenyl-2-propanol (M14) and 2-phenylpropionic acid (M16). Intraperitoneal administration of 2-phenylpropionic acid to rats showed 64% of the dose was excreted in urine as 2-phenylpropionylglucuronide (M10), 17% as unchanged 2-phenylpropionic acid, and only 0.5% as 2-phenylpropionylglycine (M11) (Dixon et al., 1977). Therefore, 2-phenylpropionic acid (M16) derived from metabolism of cumene would likely be converted to a glucuronide conjugate (M10) in rats.

M11 with an HPLC retention time of 22.3 min was present in mouse urine but not in rat urine. It was not hydrolyzed by sulfatase or β-glucuronidase. M11 had a molecular weight of 207, equivalent to 2-phenylpropionic acid (150) + glycine (75) − H2O. MS fragmentation shows loss of one glycine (208–133). In order to confirm the proposed structure, M11 was independently synthesized by converting 2-phenylpropionic acid to 2-phenylpropionyl chloride followed by reaction with glycine. A product with the retention time at 22.0 min was collected from HPLC. MS and NMR analysis confirmed that the synthetic product was 2-phenylpropionylglycine. The synthetic 2-phenylpropionylglycine had an identical retention time and mass spectra as those of M11. This metabolite was identified as 2-phenylpropionylglycine (M11).

M12 and M13 eluted at ~23.3 min. M12 eluted slightly earlier than M13, but the two metabolites usually were not totally resolved. The molecular weight of M12 was 297, equivalent to AMS (118) + [O] + N-acetylcysteine (163). MS fragmentation to 2-hydroxy-2-phenylpropanthiolic anion (m/z 167) and 2-acetylaminoacylate (m/z 128) was consistent with an
N-acetylcysteine attached at the \(\beta\)-methylene carbon (1-position) of the isopropyl side chain. MS fragmentation would give an N-acetylcysteine anion (m/z 162) if the N-acetylcysteine were attached at the \(\alpha\)-carbon (a tertiary carbon). The metabolite was likely S-(2-hydroxy-2-phenylpropyl)-N-acetylcysteine, a metabolite identified in the urine of male rats dosed with AMS (De Costa et al., 2001). Generally, \(M_{12}\) was more abundant in rat urine, and only a trace amount was observed in mouse urine. \(M_{12}\) was tentatively identified as S-(2-hydroxy-2-phenylpropyl)-N-acetylcysteine.

MS analysis showed that \(M_{13}\) had a molecular weight of 312, consistent with formation of a monohydroxycumene glucuronide (cumene (120) + [O] + glucuronide (176)). \(\beta\)-Glucuronidase hydrolysis of this glucuronide metabolite gave an aglycone with an HPLC retention time at 28.4 min, identical to that of 2-phenyl-1-propanol. \(M_{13}\) was tentatively identified as 2-phenyl-1-propanol glucuronide.

Three minor metabolites with retention times of 27.6 min, 28.4 min, and 29.5 min were occasionally observed in rat or mouse urine. These metabolites had identical retention times to those of authentic 2-phenyl-2-propanol (27.6 min, \(M_{14}\)), 2-phenyl-1-propanol (28.4 min, \(M_{15}\)), and 2-phenylpropionic acid (29.5 min, \(M_{16}\)).

Most of these metabolites (\(M_1 - 16\)) were also detected in urine from animals treated with lower doses of cumene. The percentage of dose of \(M_1 - 16\) in male rat urine from all treatment groups is shown in Table 5. \(M_9\) and \(M_{10}\) co-eluted and were counted together. \(\beta\)-Glucuronidase hydrolysis revealed that \(M_{10}\) was only a minor metabolite (Figure 4D); therefore, \(M_9\) was the most abundant metabolite in rat urine (38–50% of all radiolabeled peaks). \(M_7\) and \(M_8\) each constituted 11–20% of all radiolabeled peaks. The percentage of all other metabolites in male rat urine was less than 10% except \(M_3\), which accounted for 11% of all radiolabeled
peaks in urine from the 149 mg/kg oral dose. **M12** and **M13** were not totally resolved in most HPLC analyses and, therefore, were quantified together. The only exception was in urine of male rats dosed orally with 1.5 mg/kg where **M12**, equivalent to 2.0 ± 0.2% (n = 3) of all radiolabeled peaks, was resolved from **M13**. The percentages of the metabolites in mouse urine from all treatment groups is shown in Table 6. **M9** accounted for 30–43% of all radiolabeled peaks in mouse urine. **M8** constituted 11–20% of all radiolabeled peaks. The percentage of **M7** and **M5** ranged from 6–17% and 3–19%, respectively. Only a trace amount of **M12** was observed in mouse urine after **M13** was hydrolyzed by β-glucuronidase (data not shown).

**Biliary Metabolites**

BDC male rats received 2.1 mg/kg by IV injection and the bile was collected for 24 h. Biliary excretion accounted for 37% of the dose within 24 h of dosing. The bile collected 0–6 h was analyzed by HPLC to reveal several radiolabeled peaks (Figure 4E). Cumene was not observed in bile (data not shown). **M9** was the most abundant biliary metabolite. **M6**, **M7**, **M13**, and **M16** also were detected in male rat bile. **M16** is a low-molecular-weight metabolite and would not be expected to be excreted in bile; its presence might be due to decomposition of its glucuronide (**M10**). The characterization of cumene-derived biliary metabolites was based on comparison of their HPLC retention times with those of urinary metabolites. The quantification of cumene metabolites in male rat bile is shown in Table 5.

**In Vitro Microsomal Incubations**

Three metabolites, **M14**, **M15**, and AMS, were detected in the microsomal incubations (Figure 6). Female mouse lung microsomes (Figure 6A) metabolized more cumene than female
mouse liver microsomes (Figure 6B), female rat lung microsomes (Figure 6C), or female rat liver microsomes (Figure 6D). The percentage of these cumene metabolites in microsomal incubations is shown in Table 7.
DISCUSSION

The present study demonstrated that cumene was absorbed following oral administration to male rats and mice of both sexes and excreted primarily in urine. The excretion of $^{14}$C as VOCs was dependent on dose, sex, species, and route of administration. Expiration of $^{14}$C VOCs increased at the high doses, especially in mice, implying saturation of specific metabolic pathways. Female mice excreted more $^{14}$C as VOCs and CO$_2$ ($p = 0.05$) and retained more $^{14}$C in tissues ($p = 0.0001$) than did males at the high dose indicating male mice metabolized cumene more efficiently than females. More $^{14}$C was excreted as VOCs following an IV dose versus the comparable oral dose ($p = 0.002$).

The substantial amount of $^{14}$C in the intestines 24 h following IV injection in rats suggested biliary excretion of cumene and/or its metabolites. This was confirmed in BDC rats following excretion of 37% of a total cumene dose in bile within 24 h post-dosing. Because little $^{14}$C was excreted in feces in any treatment group, enterohepatic circulation of cumene and/or metabolites and subsequent excretion in urine is implied.

Tissue concentrations of $^{14}$C were higher in rats than in mice receiving similar doses ($p = 0.006$ for 14 mg/kg rat vs. 10 mg/kg male and female mouse oral dosing). The $^{14}$C concentrations in the kidney of male rats were much higher than in mice at comparable doses ($p < 0.0001$ for 14 mg/kg rat vs. 10 mg/kg male and female mouse oral dosing) and may indicate binding of cumene and/or metabolites to male rat-specific $\alpha_{2u}$-globulin in the kidneys (Strasser Jr. et al., 1988; Lehman-McKeeman et al., 1990). This mechanism of action may correlate with the higher incidence of renal carcinoma observed in the kidney of cumene-treated male rats in previous toxicity studies (NTP, 2009). After seven consecutive daily doses, the tissue with the highest $^{14}$C concentration in mouse was the lung which correlates with the higher incidence of
alveolar/bronchiolar adenoma and carcinoma observed in lungs of cumene-treated mice in the previous toxicity studies (NTP, 2009).

The present study reveals two previously unknown metabolic pathways of cumene: formation of AMS and ring oxidation. A proposed metabolic scheme for cumene is shown in Figure 5. 2-phenyl-1-propanol glucuronide (M13) and 2-phenyl-2-propanol glucuronide (M9) were the most abundant metabolites observed in these studies. M14 was ring-oxidized and excreted in urine as sulfate conjugates 2-(2-hydroxy-2-propyl)phenylsulfate (M2) and 4-(2-hydroxy-2-propyl)phenylsulfate (M3). M14 is a labile molecule that can dehydrate to AMS, which is excreted in the expired air or further oxidized to AMS oxide with further metabolism resulting in a sulfate (M5), glucuronides (M6 and M7), or oxidation to form an 2-hydroxy-2-phenylpropionic acid (M8).

There are two pathways for the formation of 2-phenylpropionic acid (M16): oxidation of 2-phenyl-1-propanol (M14) or oxidation of 2-phenylpropionaldehyde generated from rearrangement of AMS oxide. The stereochemistry of urinary metabolites from cumene-treated rabbits suggested that S-(+)-2-phenylpropionic acid was not from oxidation of R-(+)-2-phenyl-1-propanol (Ishida and Matsumoto, 1992). Rapid rearrangement of AMS oxide to phenylpropionaldehyde was observed in this and other studies (Rosman et al., 1986); therefore, formation of M16 from this pathway is highly feasible. However, R-(−)-2-phenylpropionic acid has been shown to partially isomerize to its S-(+)-isomer in rats (Yamaguchi and Nakamura, 1985); therefore, formation of M16 from oxidation of M14 cannot be ruled out. 2-Phenylpropionic acid (M16) was further metabolized to a glucuronide conjugate (M10), predominantly in rats, and a glycine conjugate (M11), predominantly in mice. M11 was more abundant in male mice than females, especially at the high dose.
Both *in vivo* and *in vitro* studies demonstrate that an important metabolic pathway of cumene is formation of AMS.Expiration becomes a significant excretion pathway as the cumene dose level increases. HPLC analysis of the expired VOCs of female and male mice treated with high oral doses of cumene (1064 mg/kg and 1000 mg/kg, respectively) indicated that AMS accounted for 3–4% of the total radioactivity in the expired VOCs with the rest being cumene. Only a trace amount of AMS was observed in the expired VOC of male rats.

The lung was a target organ of cumene in mice but not in rats in previous studies following inhalation exposure (NTP, 2009). Because female mice were more susceptible, cumene-metabolizing activity was studied in female mouse lung and liver microsomes and compared with female rat lung and liver microsomes. The results are shown in Figure 6 and Table 7. Female mouse lung microsomes were the most efficient in metabolizing cumene to 2-phenyl-2-propanol (M14), 2-phenyl-1-propanol (M15), and AMS. A previous study found AMS was more lethal to female mice than male mice and rats of both sexes; however, the mechanism of AMS toxicity in mice was not investigated (Morgan et al., 1999).

All *in vivo* metabolites of cumene from the AMS pathway were derived from AMS oxide. AMS oxide is mutagenic in Salmonella assays (Rosman et al., 1986) and reacts with GSH, forming a mercapturic acid conjugate (M12) excreted in urine. Therefore, AMS oxide might play a role in the higher incidence of alveolar/bronchiolar adenoma and carcinoma observed in the lung of cumene-treated mice in the NTP toxicity studies. The *in vitro* microsomal incubation study demonstrated that mouse lung converted cumene to AMS and M14, the latter of which could dehydrate to give AMS or be further oxidized. These results may help explain accumulation of $^{14}$C in mouse lung following multiple doses of $[^{14}$C]cumene, and they may correlate with the carcinogenicity of cumene in mouse but not rat lung. Styrene, which
is both pneumotoxic and hepatotoxic in mice, but not in rats, is metabolized to styrene oxide at a rate several-fold higher in Clara cells isolated from mouse lung than from rat lung (Hynes et al., 1999). CYP2F2, which has a higher activity in the Clara cells of mouse lung than the orthologous CYP2F4 in rat lung, is the primary cytochrome P450 involved in the oxidation of styrene (Hynes et al., 1999, Buckpitt et al., 1995). Further, it has been demonstrated that for coumarin, naphthalene, and styrene, which are structurally related to cumene, inhibition of CYP2F2 results in inhibition of lung toxicity (Cruzan et al., 2009 and references therein). CYP2F4 is much less prevalent in rat Clara cells and, moreover, human lungs contain much fewer Clara cells and the relevant CYP2F isoform (CYP2F1) than rats or mice (Stott et al., 2003). A cytotoxicity-driven mode of action pertaining to mouse specific lung tumors for this group of compounds by the CYP2F family recently has been proposed (Cruzan et al., 2009). These data indicate that cumene alveolar/bronchiolar cytotoxicity in humans would be much less than in mice or even rats that have not shown evidence of lung cytotoxicity.

AMS exposure also has resulted in increased accumulation of hyaline droplets in the renal tubules of male rats (Morgan et al., 1999). Hyaline droplets, which contain α-2u-globulins, can lead to granular casts and single cell necrosis, increased cell division and tubule hyperplasia, and finally renal tubule adenoma and carcinoma (Rodgers et al., 1993). If the above proposed tumorigenicity pathway is correct, it follows that cumene would not be considered a renal tumor risk in humans as α-2u-globulin is a male rat-specific protein that is not present in female rats, male or female mice, or humans (Flamm and Lehman-McKeeman, 1991; Lehman-McKeeman, 1993; Lehman-McKeeman and Caudill, 1992; Swenberg, 1993).

Other possible reactive metabolites include the arene oxide intermediates from ring-oxidation of 2-phenyl-2-propanol (M14) to 2-(2-hydroxy-2-propyl)phenol and 4-(2-hydroxy-2-
propyl)phenol. In addition, further multiple oxidation of these two phenols can lead to a catechol and subsequent quinonemethide (Figure 5), the latter of which are known to be reactive toward biomolecules (Liu et al., 2005).

In summary, the present work has provided a comprehensive investigation of the disposition and metabolism of cumene in male F344 rats and B6C3F1 mice of both sexes and an additional study of cumene metabolism in microsomes. The excretion data for the rat supports enterohepatic circulation of cumene and/or its metabolites. Also, the male rat had the highest concentrations of cumene in the kidney tissues, which supports previous studies implicating binding of cumene, AMS, and/or other metabolites to male rat-specific α2u-globulin in the kidney that correlates with the higher incidence of renal tubule adenoma and carcinoma in the male rat. This mechanism of adenoma and carcinoma in rat kidney may not be pertinent to humans. In the mouse, the lungs contained the highest concentration of 14C after 7 consecutive daily doses, which correlate with the higher incidence of alveolar/bronchiolar adenoma and carcinoma observed in lungs of cumene-treated mice (NTP, 2009). This mechanism of adenoma and carcinoma in mouse lung also may not be pertinent to humans. The results of these studies indicate disposition and metabolism-based mechanisms that correlate with the differential carcinogenic response observed in cumene-exposed rats and mice and the decreasing relevance of these animals as models for cumene toxicity in humans.
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AUTHORSHIP CONTRIBUTION

Participated in research design: Chen, McDonald, Dix, and Sanders.
Conducted experiments: Chen, Kramer, Thomas, and McDonald.
Performed data analysis: Chen, Wegerski.
Wrote or contributed to the writing of the manuscript: Chen, Wegerski, and Sanders.
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FOOTNOTES

1. This project was conducted for the National Toxicology Program, National Institute of Environmental Health Sciences [NIEHS], National Institutes of Health, Department of Health and Human Services, under Contract No. N01-ES-75562 [HHSN291200775562C].

2. Disclaimer: This article may be the work product of an employee or group of employees of the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), however, the statements, opinions or conclusions contained therein do not necessarily represent the statements, opinions or conclusions of NIEHS, NIH, or the United States government.
FIGURE LEGENDS

Figure 1. Chemical structure, formula, and molecular weight of $^{14}$C]cumene.

Figure 2. Cumene-derived radioactivity (nmol-eq/g tissue) in blood, liver, kidney, and lung of (A) male rats 24 h after 1, 3, or 7 daily oral doses (14 mg/kg) of $^{14}$C]cumene and (B) female mice 24 h after 1, 3, or 7 daily oral doses (150 mg/kg) of $^{14}$C]cumene. Data are expressed as mean ± S.D. of 4 animals/treatment group.

Figure 3. Representative HPLC radio- and UV (254 nm)-chromatograms of cumene and AMS in the expired air (0–6 h) of a male mouse dosed orally with a single 1000 mg/kg dose.

Figure 4. Representative HPLC radiochromatograms of cumene metabolites (M1–16) in (A) urine (0–24 h) of a male rat dosed orally with a single 140 mg/kg dose, (B) in urine (0–24 h) of a male mouse dosed orally with a single 1000 mg/kg dose, (C) in urine (0–24 h) of a female mouse dosed orally with a single 1000 mg/kg dose, (D) in glucuronidase/sulfatase-hydrolyzed urine (0–24 h) of a male rat dosed orally with a single 140 mg/kg dose, and (E) in bile (0–6 h) of a BDC male rat dosed intravenously with a 1.4 mg/kg single dose.

Figure 5. Proposed metabolic pathways of cumene.

Figure 6. Representative HPLC radiochromatograms of cumene and metabolites in (A) female mouse lung, (B) female mouse liver, (C) female rat lung, and (D) female rat liver microsomal incubations (100 µL each).
### TABLE 1

**Target Dose (mg/kg) of Cumene in Disposition Studies**

<table>
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<tr>
<th>Dose Route</th>
<th>Male Rat</th>
<th>Male</th>
<th>Female</th>
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<tr>
<td>IV</td>
<td>1.4</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Oral</td>
<td>1.4, 14, 140</td>
<td>10, 50, 100, 1000</td>
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### TABLE 2

Recovery of $^{14}$C following Administration of Cumene to Male Rats and Mice of Both Sexes$^a$

<table>
<thead>
<tr>
<th>Route</th>
<th>Frequency $^b$</th>
<th>Dose (mg/kg)</th>
<th>Time (h)</th>
<th>% Dose in Male Rat</th>
<th>% Dose in Female Mouse</th>
<th>Total recovery $^c$</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Urine</td>
<td>Feces</td>
<td>G.I. Tract</td>
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<tr>
<td>IV</td>
<td>S</td>
<td>1.4</td>
<td>24</td>
<td>90.1 ± 5.9</td>
<td>1.2 ± 0.1</td>
<td>4.6 ± 0.9</td>
</tr>
<tr>
<td>Oral</td>
<td>S</td>
<td>1.4</td>
<td>24</td>
<td>77.4 ± 10.1</td>
<td>1.2 ± 0.5</td>
<td>1.3 ± 0.9</td>
</tr>
<tr>
<td>Oral</td>
<td>S</td>
<td>14</td>
<td>24</td>
<td>70.2 ± 3.4</td>
<td>1.2 ± 0.8</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Oral</td>
<td>S</td>
<td>140</td>
<td>24</td>
<td>69.8 ± 8.9</td>
<td>0.9 ± 0.1</td>
<td>8.7 ± 5.0</td>
</tr>
<tr>
<td>Oral</td>
<td>S</td>
<td>14</td>
<td>72</td>
<td>85.6 ± 4.0</td>
<td>5.3 ± 1.8</td>
<td>ND</td>
</tr>
<tr>
<td>Oral</td>
<td>R</td>
<td>14 x 3</td>
<td>24</td>
<td>73.9 ± 2.7</td>
<td>2.0 ± 0.7</td>
<td>ND</td>
</tr>
<tr>
<td>Oral</td>
<td>R</td>
<td>14 x 7</td>
<td>24</td>
<td>81.3 ± 5.6</td>
<td>1.5 ± 1.1</td>
<td>ND</td>
</tr>
<tr>
<td>IV</td>
<td>S</td>
<td>10</td>
<td>24</td>
<td>47.4 ± 6.5</td>
<td>1.8 ± 1.8</td>
<td>2.2 ± 1.0</td>
</tr>
<tr>
<td>Oral</td>
<td>S</td>
<td>10</td>
<td>24</td>
<td>105.0 ± 5.0</td>
<td>4.5 ± 1.0</td>
<td>3.0 ± 3.5</td>
</tr>
<tr>
<td>Oral</td>
<td>S</td>
<td>50</td>
<td>24</td>
<td>90.2 ± 6.1</td>
<td>5.3 ± 4.0</td>
<td>4.5 ± 5.6</td>
</tr>
<tr>
<td>Oral</td>
<td>S</td>
<td>100</td>
<td>24</td>
<td>79.3 ± 9.9</td>
<td>4.0 ± 2.3</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Oral</td>
<td>S</td>
<td>1000</td>
<td>24</td>
<td>80.9 ± 6.1</td>
<td>5.2 ± 4.4</td>
<td>13.9 ± 3.7</td>
</tr>
<tr>
<td>IV</td>
<td>S</td>
<td>10</td>
<td>24</td>
<td>74.0 ± 8.4</td>
<td>1.7 ± 0.6</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>Oral</td>
<td>S</td>
<td>10</td>
<td>24</td>
<td>93.7 ± 3.4</td>
<td>3.6 ± 1.9</td>
<td>5.2 ± 5.6</td>
</tr>
<tr>
<td>Oral</td>
<td>S</td>
<td>150</td>
<td>24</td>
<td>86.5 ± 5.0</td>
<td>2.5 ± 1.3</td>
<td>2.9 ± 0.8</td>
</tr>
<tr>
<td>Oral</td>
<td>S</td>
<td>1000</td>
<td>24</td>
<td>79.2 ± 5.7</td>
<td>2.0 ± 0.2</td>
<td>21.9 ± 3.6</td>
</tr>
<tr>
<td>Oral</td>
<td>R</td>
<td>150 x 3</td>
<td>24</td>
<td>80.1 ± 11.1</td>
<td>4.7 ± 1.4</td>
<td>ND</td>
</tr>
<tr>
<td>Oral</td>
<td>R</td>
<td>150 x 7</td>
<td>24</td>
<td>77.2 ± 7.8</td>
<td>4.7 ± 1.4</td>
<td>ND</td>
</tr>
</tbody>
</table>

$^a$Data are expressed as mean ± SD of 4 animals/treatment group.

$^b$Frequency of dosing: S = single dosing, R = repeated daily dosing.

$^c$The animals were sacrificed 24 or 72 h after single dosing or 24 h after the last of 3 or 7 consecutive daily doses.

$^d$Stomach, small and large intestines, and their contents.

$^e$Includes $^{14}$CO$_2$ collected in some studies and all surveyed tissues listed in the Methods section, including the digested carcasses.

$^f$Not Determined.
### TABLE 3

Distribution of $^{14}$C in Selected Tissues following Administration of Single Doses of Cumene to Male Rats and Mice of Both Sexes$^a$

<table>
<thead>
<tr>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>Time (h)</th>
<th>Blood Conc. (nmol-eq/g)</th>
<th>Liver Conc. (nmol-eq/g)</th>
<th>T/B Ratio$^b$</th>
<th>Kidney Conc. (nmol-eq/g)</th>
<th>T/B Ratio</th>
<th>Lung Conc. (nmol-eq/g)</th>
<th>T/B Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>1.4</td>
<td>24</td>
<td>0.17 ± 0.03</td>
<td>1.1 ± 0.3</td>
<td>6.2 ± 1.5</td>
<td>1.3 ± 0.3</td>
<td>7.9 ± 0.9</td>
<td>0.24 ± 0.02</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Oral</td>
<td>1.4</td>
<td>24</td>
<td>0.37 ± 0.10</td>
<td>2.2 ± 0.6</td>
<td>5.9 ± 0.5</td>
<td>2.8 ± 1.2</td>
<td>7.8 ± 3.2</td>
<td>0.33 ± 0.09</td>
<td>0.91 ± 0.22</td>
</tr>
<tr>
<td>Oral</td>
<td>14</td>
<td>24</td>
<td>3.8 ± 0.3</td>
<td>22 ± 3</td>
<td>5.9 ± 1.4</td>
<td>33 ± 3</td>
<td>8.6 ± 1.7</td>
<td>6.1 ± 2.6</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td>Oral</td>
<td>140</td>
<td>24</td>
<td>35 ± 6</td>
<td>146 ± 38</td>
<td>4.1 ± 0.6</td>
<td>279 ± 30</td>
<td>8.0 ± 0.7</td>
<td>26 ± 6</td>
<td>0.74 ± 0.05</td>
</tr>
<tr>
<td>Oral</td>
<td>14</td>
<td>72</td>
<td>0.30 ± 0.07</td>
<td>1.1 ± 0.3</td>
<td>3.8 ± 0.5</td>
<td>1.4 ± 0.5</td>
<td>4.5 ± 0.8</td>
<td>0.48 ± 0.05</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>IV</td>
<td>10</td>
<td>24</td>
<td>0.13 ± 0.03</td>
<td>0.67 ± 0.11</td>
<td>5.2 ± 0.5</td>
<td>0.66 ± 0.03</td>
<td>5.2 ± 1.1</td>
<td>1.1 ± 0.1</td>
<td>9.1 ± 2.5</td>
</tr>
<tr>
<td>Oral</td>
<td>10</td>
<td>24</td>
<td>0.40 ± 0.04</td>
<td>2.6 ± 0.8</td>
<td>6.5 ± 1.6</td>
<td>3.2 ± 1.2</td>
<td>8.1 ± 3.4</td>
<td>1.4 ± 0.9</td>
<td>3.6 ± 2.5</td>
</tr>
<tr>
<td>Oral</td>
<td>50</td>
<td>24</td>
<td>0.61 ± 0.18</td>
<td>4.5 ± 0.8</td>
<td>7.8 ± 2.6</td>
<td>3.0 ± 1.1</td>
<td>5.3 ± 2.3</td>
<td>2.5 ± 0.6</td>
<td>4.4 ± 1.8</td>
</tr>
<tr>
<td>Oral</td>
<td>100</td>
<td>24</td>
<td>1.8 ± 0.2</td>
<td>7.4 ± 4.6</td>
<td>4.3 ± 2.8</td>
<td>7.9 ± 5.2</td>
<td>4.6 ± 3.0</td>
<td>5.8 ± 3.2</td>
<td>3.3 ± 1.9</td>
</tr>
<tr>
<td>Oral</td>
<td>1000</td>
<td>24</td>
<td>9.4 ± 2.1</td>
<td>41 ± 9</td>
<td>4.4 ± 0.4</td>
<td>71 ± 35</td>
<td>7.3 ± 2.2</td>
<td>21 ± 9</td>
<td>2.4 ± 1.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>Time (h)</th>
<th>Blood Conc. (nmol-eq/g)</th>
<th>Liver Conc. (nmol-eq/g)</th>
<th>T/B Ratio$^b$</th>
<th>Kidney Conc. (nmol-eq/g)</th>
<th>T/B Ratio</th>
<th>Lung Conc. (nmol-eq/g)</th>
<th>T/B Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>10</td>
<td>24</td>
<td>0.24 ± 0.08</td>
<td>1.3 ± 0.4</td>
<td>5.3 ± 0.7</td>
<td>0.58 ± 0.20</td>
<td>2.4 ± 0.1</td>
<td>1.7 ± 0.6</td>
<td>6.9 ± 0.9</td>
</tr>
<tr>
<td>Oral</td>
<td>10</td>
<td>24</td>
<td>0.44 ± 0.04</td>
<td>2.4 ± 0.0</td>
<td>5.5 ± 0.4</td>
<td>0.93 ± 0.13</td>
<td>2.1 ± 0.3</td>
<td>0.85 ± 0.18</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>Oral</td>
<td>50</td>
<td>24</td>
<td>1.8 ± 0.5</td>
<td>7.6 ± 1.7</td>
<td>4.7 ± 2.1</td>
<td>4.0 ± 0.9</td>
<td>2.3 ± 0.4</td>
<td>7.9 ± 1.2</td>
<td>4.6 ± 0.8</td>
</tr>
<tr>
<td>Oral</td>
<td>1000</td>
<td>24</td>
<td>38 ± 15</td>
<td>101 ± 32</td>
<td>2.7 ± 0.4</td>
<td>139 ± 109</td>
<td>4.0 ± 2.6</td>
<td>101 ± 57</td>
<td>2.6 ± 0.6</td>
</tr>
</tbody>
</table>

$^a$Each value represents the mean ± SD of 3–4 animals/treatment group.

$^b$Tissue-to-blood ratio.
### TABLE 4
Cumene-derived Radioactivity in All Surveyed Tissues 24 h following Single or Repeat Dosing\(^a\) of 14 mg/kg to Male Rats and 150 mg/kg to Female Mice

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 Dose</th>
<th>3 Doses</th>
<th>7 Doses</th>
<th>1 Dose</th>
<th>3 Doses</th>
<th>7 Doses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. (nmol-eq/g)</td>
<td>T/B Ratio</td>
<td>Conc. (nmol-eq/g)</td>
<td>T/B Ratio</td>
<td>Conc. (nmol-eq/g)</td>
<td>T/B Ratio</td>
</tr>
<tr>
<td>Adipose</td>
<td>12 ± 7</td>
<td>3.1 ± 1.7</td>
<td>14 ± 9</td>
<td>2.6 ± 2.0</td>
<td>9.0 ± 7.4</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>Urinary Bladder</td>
<td>72 ± 38</td>
<td>19 ± 12</td>
<td>971 ± 1126</td>
<td>152 ± 154</td>
<td>106 ± 81</td>
<td>14 ± 10</td>
</tr>
<tr>
<td>Blood</td>
<td>3.8 ± 0.3</td>
<td>unity</td>
<td>5.8 ± 1.1(^a)</td>
<td>unity</td>
<td>6.3 ± 2.7</td>
<td>unity</td>
</tr>
<tr>
<td>Brain</td>
<td>1.4 ± 0.2</td>
<td>0.37 ± 0.04</td>
<td>3.2 ± 1.2</td>
<td>0.56 ± 0.25</td>
<td>2.1 ± 1.0</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>Heart</td>
<td>2.4 ± 0.3</td>
<td>0.63 ± 0.06</td>
<td>4.0 ± 1.2</td>
<td>0.69 ± 0.10</td>
<td>4.0 ± 1.6</td>
<td>0.63 ± 0.03</td>
</tr>
<tr>
<td>Kidney</td>
<td>33 ± 3</td>
<td>8.6 ± 1.7</td>
<td>60 ± 27</td>
<td>10 ± 4</td>
<td>57 ± 19</td>
<td>9.5 ± 1.8</td>
</tr>
<tr>
<td>Liver</td>
<td>22 ± 3</td>
<td>5.9 ± 1.4</td>
<td>31 ± 9</td>
<td>5.4 ± 1.1</td>
<td>32 ± 21</td>
<td>4.8 ± 1.2</td>
</tr>
<tr>
<td>Lung</td>
<td>6.1 ± 2.6</td>
<td>1.6 ± 0.6</td>
<td>6.6 ± 1.8</td>
<td>1.1 ± 0.1</td>
<td>7.4 ± 2.3</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.0 ± 0.5</td>
<td>0.27 ± 0.13</td>
<td>2.8 ± 0.1(^a)</td>
<td>0.49 ± 0.07</td>
<td>1.9 ± 1.4</td>
<td>0.29 ± 0.16</td>
</tr>
<tr>
<td>Skin</td>
<td>2.5 ± 0.5</td>
<td>0.66 ± 0.17</td>
<td>5.8 ± 1.8(^a)</td>
<td>1.0 ± 0.3</td>
<td>7.3 ± 2.9(^a)</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.4 ± 0.8</td>
<td>0.62 ± 0.17</td>
<td>5.4 ± 1.4(^a)</td>
<td>0.94 ± 0.23</td>
<td>5.7 ± 3.2</td>
<td>0.91 ± 0.35</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.0 ± 6.5</td>
<td>0.1 ± 1.8</td>
<td>3.8 ± 1.9</td>
<td>0.63 ± 0.22</td>
<td>2.9 ± 2.9</td>
<td>0.49 ± 0.61</td>
</tr>
<tr>
<td>Testes</td>
<td>1.3 ± 0.4</td>
<td>0.33 ± 0.12</td>
<td>9.1 ± 7.0</td>
<td>1.6 ± 1.3</td>
<td>1.8 ± 1.1</td>
<td>0.28 ± 0.05</td>
</tr>
</tbody>
</table>

\(^a\)Consecutive daily doses.
\(^b\)Tissue-to-blood ratio.
\(^c\)Each values represents the mean ± standard deviation of 4 animals/treatment group.
\(^d\)Not detected: the average concentration was <0 after background correction.
\(^e\)Not applicable.
\(^f\)Statistically higher (p ≤ 0.05) in the same tissues from 3 or 7 dose studies versus the single-dose study.
\(^g\)Statistically higher (p ≤ 0.05) in the same tissues from the 7 dose study versus the 3-dose study.
### TABLE 5

Percentage of Dose of Cumene Metabolites\(^\text{a}\) in Cumulative 0–24 h Male Rat Urine and 0–6 h Male Rat Bile

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Normal Male Rats</th>
<th>BDC-MR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>140 mg/kg po Urine</td>
<td>14 mg/kg po Urine</td>
</tr>
<tr>
<td>M1</td>
<td>ND(^\text{c})</td>
<td>ND</td>
</tr>
<tr>
<td>M2</td>
<td>Trace(^d)</td>
<td>Trace</td>
</tr>
<tr>
<td>M3</td>
<td>11.4 ± 1.6</td>
<td>8.2 ± 0.9</td>
</tr>
<tr>
<td>M4</td>
<td>5.6 ± 0.5</td>
<td>5.2 ± 0.7</td>
</tr>
<tr>
<td>M5</td>
<td>2.6 ± 0.3</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>M6</td>
<td>1.6 ± 0.0</td>
<td>ND(^c)</td>
</tr>
<tr>
<td>M7</td>
<td>17.8 ± 1.0</td>
<td>20.1 ± 0.6</td>
</tr>
<tr>
<td>M8</td>
<td>16.4 ± 2.0</td>
<td>12.1 ± 0.8</td>
</tr>
<tr>
<td>M9+M10</td>
<td>38.1 ± 2.2</td>
<td>47.0 ± 1.3</td>
</tr>
<tr>
<td>M11</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M12+M13</td>
<td>4.8 ± 0.5</td>
<td>4.9 ± 0.5</td>
</tr>
<tr>
<td>M14</td>
<td>1.6(^c)</td>
<td>Trace</td>
</tr>
<tr>
<td>M15</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M16</td>
<td>2.1 ± 0.5</td>
<td>Trace</td>
</tr>
</tbody>
</table>

\(^a\)Percent of all radiolabeled peaks; mean ± SD; n = 4.

\(^b\)M10 is a minor metabolite co-eluted with M9.

\(^c\)Not detected.

\(^d\)A trace amount was observed but not quantified.

\(^e\)n = 1.

\(^f\)n = 2.

\(^g\)n = 3.
TABLE 6
Percentage of Dose of Cumene Metabolites<sup>a</sup> in Cumulative 0–24 h Mouse Urine

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>1000 mg/kg po</th>
<th>100 mg/kg po</th>
<th>10 mg/kg po</th>
<th>1000 mg/kg po</th>
<th>150 mg/kg po</th>
<th>10 mg/kg po</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male Mice</td>
<td>Female Mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>Urine</td>
<td>Urine</td>
<td>Urine</td>
<td>Urine</td>
<td>Urine</td>
</tr>
<tr>
<td>M1</td>
<td>Trace&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>3.0 ± 0.3</td>
<td>2.9 ± 1.0</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>M2</td>
<td>Trace</td>
<td>ND</td>
<td>ND</td>
<td>4.4 ± 1.0</td>
<td>3.4 ± 0.2</td>
<td>ND</td>
</tr>
<tr>
<td>M3</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>Trace</td>
<td>Trace</td>
<td>ND</td>
</tr>
<tr>
<td>M4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Trace</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M5</td>
<td>3.0 ± 0.1</td>
<td>6.3 ± 0.6</td>
<td>8.4 ± 0.3</td>
<td>5.8 ± 1.3</td>
<td>16.7 ± 1.9</td>
<td>19.1 ± 1.4</td>
</tr>
<tr>
<td>M6</td>
<td>4.4 ± 0.4</td>
<td>2.9 ± 0.4</td>
<td>3.1 ± 0.8</td>
<td>4.2 ± 0.5</td>
<td>2.6 ± 0.7</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>M7</td>
<td>16.9 ± 0.5</td>
<td>11.9 ± 0.4</td>
<td>8.6 ± 0.9</td>
<td>16.5 ± 1.3</td>
<td>9.0 ± 1.8</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>M8</td>
<td>12.8 ± 2.2</td>
<td>14.4 ± 0.8</td>
<td>15.7 ± 0.9</td>
<td>20.4 ± 0.8</td>
<td>12.9 ± 0.7</td>
<td>11.4 ± 0.9</td>
</tr>
<tr>
<td>M9</td>
<td>42.8 ± 2.4</td>
<td>39.3 ± 3.4</td>
<td>33.5 ± 2.2</td>
<td>36.8 ± 2.1</td>
<td>35.1 ± 2.1</td>
<td>29.8 ± 0.9</td>
</tr>
<tr>
<td>M11</td>
<td>11.0 ± 0.5</td>
<td>6.4 ± 1.3</td>
<td>5.1 ± 0.9</td>
<td>2.8 ± 0.2</td>
<td>2.9 ± 0.4</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>M12&lt;sup&gt;e&lt;/sup&gt;+M13</td>
<td>5.8 ± 1.5</td>
<td>2.1 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>2.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.3 ± 0.4</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>M14</td>
<td>ND</td>
<td>1.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M15</td>
<td>ND</td>
<td>1.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M16</td>
<td>Trace</td>
<td>ND</td>
<td>ND</td>
<td>Trace</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup>Percent of all radiolabeled peaks; mean ± SD; n = 4.

<sup>b</sup>Only a trace amount of M12 was observed in mouse urine.

<sup>c</sup>A trace amount was observed but not quantified.

<sup>d</sup>Not detected.

<sup>e</sup>n = 1.
**TABLE 7**

Percentage of Cumene and Metabolites\(^a\) in Microsomal Incubations

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Female Mouse</th>
<th>Female Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lung</td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td>Microsomes</td>
<td>Microsomes</td>
</tr>
<tr>
<td>2-Phenyl-2-propanal</td>
<td>37.7 ± 2.3</td>
<td>15.5 ± 3.3</td>
</tr>
<tr>
<td>(M14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Phenyl-1-propanal</td>
<td>3.4 ± 0.8</td>
<td>ND(^b)</td>
</tr>
<tr>
<td>(M15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMS</td>
<td>6.2 ± 0.5</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>Cumene</td>
<td>52.8 ± 2.4</td>
<td>81.2 ± 3.4</td>
</tr>
</tbody>
</table>

\(^a\)Percent of all radiolabeled peaks; mean ± SD; n = 4.

\(^b\)Not detected.

\(^c\)AMS was detected in two (1.8% and 1.1%) of four incubations.
Chemical Formula: $\text{C}_9\text{H}_{12}$
Molecular Weight: 120.2
* indicates position of $^{14}\text{C}$ label

Figure 1
Figure 4
Supplemental data for Drug Metabolism and Disposition #34769

**SUPPLEMENTAL TABLE 1**

Characterization of Cumene Metabolites

<table>
<thead>
<tr>
<th>Abbr.</th>
<th>Assignment</th>
<th>HPLC (min)</th>
<th>Spectral properties and enzymatic hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Not identified</td>
<td>10.1</td>
<td>Not hydrolyzed by sulfatase or glucuronidase. Decomposed to two peaks with HPLC retention times at 16.4 and 17.0 min after isolation. ESI(−)-MS: m/z 231 [M - H]−</td>
</tr>
<tr>
<td>M2</td>
<td>2-(2-hydroxy-2-propyl)phenylsulfate</td>
<td>13.4</td>
<td>1H NMR (D2O): δ 7.56 (d, J = 8.5 Hz, 1H, phenyl 3-H), 7.47 (t, J = 8.3 Hz, 1H, phenyl 4-H), 7.45 (t, J = 7.5 Hz, 1H, phenyl 5-H), 7.32 (d, J = 7.5 Hz, 1H, phenyl 6-H), 1.55 (s, 6H, 1,3-CH3). Hydrolyzed by sulfatase. ESI(−)-MS: m/z 231 [M - H]−, 213 [M - H - H2O]−, 151 [M - H - SO3]−, 133 [M - H - H2O - SO3]−</td>
</tr>
<tr>
<td>M3</td>
<td>4-(2-hydroxy-2-propyl)phenylsulfate</td>
<td>13.8</td>
<td>1H NMR (D2O): δ 7.59 (d, J = 9.0 Hz, 2H, phenyl 3,5-H), 7.34 (d, J = 8.5 Hz, 1H, phenyl 4-H), 7.29 (t, J = 7.5 Hz, 1H, phenyl 5-H), 7.25 (s, 6H, 1,3-CH3). Hydrolyzed by sulfatase. ESI(−)-MS: m/z 231 [M - H]−, 213 [M - H - H2O]−, 151 [M - H - SO3]−, 133 [M - H - H2O - SO3]−</td>
</tr>
</tbody>
</table>
Hz, 2H, phenyl 2,6-H), 1.62 (s, 6H, 1,3-CH₃).

Hydrolyzed by sulfatase to a peak with an HPLC retention time at 17.8 min.

Not hydrolyzed by sulfatase or glucuronidase. Decomposed to two peaks with HPLC retention times at 22.6 and 23.1 min after isolation.


1H NMR (D₂O): δ 7.53 (d, J = 8.5 Hz, 2H, phenyl 2,6-H), 7.46 (t, J = 8.5 Hz, 2H, phenyl 3,5-H), 7.37 (t, J = 6 Hz, 1H, phenyl 4-H), 4.17 (AB quartet, J = 13.5 Hz, 2H, 1-CH₂), 1.59 (s, 3H, 3-CH₃).

Hydrolyzed by sulfatase to 2-phenyl-1,2-propanediol.

ESI(−)-MS: m/z 327 [M - H]⁻.

1H NMR (D₂O): δ 7.57 (d, J = 8.5 Hz, 2H, phenyl 2,6-H), 7.42 (t, J = 6.5 Hz, 2H, phenyl 3,5-H), 7.38 (t, J = 6.0 Hz, 1H, phenyl 4-H), 4.47 (d, J = 6.5 Hz, 1H, Gluc 1'-H), 3.98 (AB quartet, J = 13.0 Hz, 2H, 1-CH₂), 3.55 (d, J = 9.0 Hz, 1H, Gluc 5'-H), 3.49 (t, J = 7.5 Hz, 1H, Gluc 2'-H), 3.43-3.37 (m, 2H, Gluc 3',4'-H), 1.62 (s, 3H, 3-CH₃).

Hydrolyzed by glucuronidase.


1H NMR (D₂O): δ 7.52 (d, J = 7.5 Hz, 2H, phenyl 2,6-H), 7.44 (t, J = 7.5 Hz, 2H, phenyl 3,5-H), 7.35 (t, J = 5.5 Hz, 1H, phenyl 4-H), 4.49 (d, J = 7.5 Hz, 1H, Gluc 1'-H), 4.19 (d, J = 11.0 Hz, 1H, Gluc 5'-H), 3.88 (AB quartet, J = 11.5 Hz, 2H, 1-CH₂), 3.56-3.47 (m, 2H, Gluc 2',4'-H), 3.30 (t, J = 8.0 Hz, 1H, Gluc 3'-H), 1.54 (s, 3H, 3-CH₃).

Hydrolyzed by glucuronidase to 2-phenyl-1,2-propanediol.


1H NMR (D₂O): δ 7.59 (d, J = 7.5 Hz, 2H, phenyl-2,6-H), 7.48 (t, J = 9.0 Hz, 2H, phenyl-3,5-H), 7.35 (t, J = 5.5 Hz, 1H, phenyl-4-H), 4.49 (d, J = 7.5 Hz, 1H, Gluc 1'-H), 4.19 (d, J = 11.0 Hz, 1H, Gluc 5'-H), 3.88 (AB quartet, J = 11.5 Hz, 2H, 1-CH₂), 3.56-3.47 (m, 2H, Gluc 2',4'-H), 3.30 (t, J = 8.0 Hz, 1H, Gluc 3'-H), 1.54 (s, 3H, 3-CH₃).
Hz, 2H, phenyl 3.5-H), 7.41 (t, J = 7.0 Hz, 1H, phenyl 4-H), 1.81 (s, 3H, 3-CH3).

Comparison with authentic 2-phenyl-2-hydroxypropionic acid.

ESI(-)-MS: m/z 311 [M - H]-, 193 [glucuronide]-.

1H NMR (D2O): δ 7.63 (d, J = 7.5 Hz, 2H, phenyl 2,6-H), 7.43 (t, J = 7.3 Hz, 2H, phenyl 3,5-H), 7.37 (t, J = 6.5 Hz, 1H, phenyl 4-H), 4.35 (d, J = 7.5 Hz, 1H, Gluc 1'-H), 3.51 (d, J = 9.0 Hz, 1H, Gluc 5'-H), 3.47 (t, J = 9.0 Hz, 1H, Gluc 2'-H), 3.35 (t, J = 7.5 Hz, 1H, Gluc 4'-H), 3.29 (t, J = 8.0 Hz, 1H, Gluc 3'-H), 1.71 (s, 3H, CH3), 1.62 (s, 3H, CH3).

Hydrolyzed by glucuronidase to 2-phenyl-2-propanol.

ESI(-)-MS: m/z 325 [M - H]-; ESI(-)-MS/MS of m/z 325: m/z 175 [glucuronide - H2O]-, 149 [M - glucuronide]-.

1H NMR (D2O): δ 1.53 (d, J = 7.0 Hz, 3H, 3-CH3); the other signals were obscured by the signals of M9.

Hydrolyzed by glucuronidase to 2-phenylpropionic acid.

ESI(-)-MS: m/z 206 [M - H]-.


Comparison with synthetic 2-phenylpropionylglycine.


ESI(-)-MS [M – H]-: m/z 311; ESI(-)-MS/MS of m/z 311: m/z 293 [M - H - H2O]-, 193 [glucuronide]-.

Hydrolyzed by glucuronidase to 2-phenyl-1-propanol.
<table>
<thead>
<tr>
<th>M14</th>
<th>2-phenyl-2-propanol</th>
<th>27.6</th>
<th>Comparison with authentic 2-phenyl-2-propanol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>M15</td>
<td>2-phenyl-1-propanol</td>
<td>28.4</td>
<td>Comparison with authentic 2-phenyl-1-propanol.</td>
</tr>
<tr>
<td>M16</td>
<td>2-phenylpropionic acid</td>
<td>29.5</td>
<td>Comparison with authentic 2-phenylpropionic acid.</td>
</tr>
</tbody>
</table>