Disposition and Metabolism of Cumene in F344 Rats and B6C3F1 Mice

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ABSTRACT:
Cumene is a high-production volume chemical that has been shown to be a central nervous system depressant and has been implicated as a long-term exposure carcinogen in experimental animals. The absorption, distribution, metabolism, and excretion of [14C]cumene (isopropylbenzene) was studied in male rats and mice of both sexes after oral or intravenous administration. In both species and sexes, urine accounted for the majority of the excretion (typically ≥70%) by oral and intravenous administration. Enterohepatic circulation of cumene and/or its metabolites was indicated because 37% of the total dose was excreted in bile in bile duct-cannulated rats with little excreted in normal rats. The highest tissue [14C] levels in rats were observed in adipose tissue, liver, and kidney with no accumulation observed after repeat dosing up to 7 days. In contrast, mice contained the highest concentrations of [14C] at 24 h after dosing in the liver, kidney, and lung, with repeat dosing accumulation of [14C] 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 major urinary and biliary metabolites identified. The volatile organics in the expired air comprised mainly cumene and up to 4% α-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) (Fig. 1) is a natural constituent of crude oil, petroleum products, and finished fuels and is used as a thinner for paints, lacquers, and enamels (Badavari, 1989; Hazardous Substances Data Bank online database, http://www.toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?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 (U.S. Environmental Protection Agency, 1988). Subsequently, there is human exposure to cumene in many occupational and environmental settings. As for other alkylbenzenes, cumene exhibits acute toxicity in animals as a central nervous system depressant (Tegeris and Balster, 1994). In humans, cumene was readily absorbed after head-only inhalation exposure (Sefczuk 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 (Hazardous Substances Data Bank online database, http://www.toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB, 2005).

Acute exposure of mice to cumene vapor (2000–8000 ppm, 20 min) produced effects similar to those of central nervous system depressant drugs (Tegeris and Balster, 1994). Cumene was negative in vivo and in vitro mutagenicity tests (except in a micronucleus assay in Fisher 344 rats, in which cumene was weakly positive at a dosage of 5000 ppm) in the National Toxicology Program, 2009). Similar results were found for coumarin (National Toxicology Program, 1993), naphthalene (National Toxicology Program, 1992, 2000), styrene (Cruzan et al., 1998, 2001), ethylbenzene (National Toxicology Program, 1999), α-methylstyrene (AMS) (National Toxicology Program, 2007a), divinylbenzene (Na-
Metabolism in female rat lung and liver microsomes was also studied for comparison.

**Materials and Methods**

**Materials.** [Ring-U-¹⁴C]cumene (specific activity 54 mCi/mmol) was obtained from Moravek Biochemicals (Brea, CA). [¹⁴C]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). Alkanulis-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 Life and Analytical Sciences (Waltham, MA). In-Flow ES scintillation cocktail was obtained from IN/US Systems (Tampa, FL). Hydrogen peroxide (H₂O₂, 30%) was purchased from Thermo Fisher Scientific (Waltham, MA). 2-Phenyl-2-propanol was purchased from Fluka (Buchs, Switzerland). 2-Phenyl-1-propanol, 2-phenyl-1,2-propanediol, 2-hydroxy-2-phenylpropionic acid, 2-phenylpropionic acid, 2-phenylpropanaldehyde, AMS, thionyl chloride, glycine, trifluoroacetic acid, dithiothreitol, heparin, β-glucuronidase from *Escherichia coli* (type VIIA, sulfatase-free), β-glucuronidase/sulfatase from *Helix pomatia* (type H-1), glucose 6-phosphate, glucose-6-phosphate dehydrogenase, and NADP⁺ were obtained from Sigma-Aldrich (St. Louis, MO). AMS oxide was purchased from TCI America (Portland, OR). The BCA Protein Assay Kit and albumin standard were purchased from Pierce Chemical (Rockford, IL).

**Instruments.** HPLC analyses were performed on an Agilent (Santa Clara, CA) 1100 high-performance liquid chromatograph and an IN/US Systems β-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 was delivered in a 3:1 scintillation/eluion ratio. A Luna C18 column (5 μm, 4.6 x 150 mm; Phenomenex, Torrance, CA) was used for dispostion studies. The mobile phase included H₂O (solvent A) and acetoni-trile (solvent B). The elution began with a linear gradient from 100% B to 95% B over 15 min and was 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.

An Inertsil C8 column (5 μm, 4.6 x 250 mm; Varian, Inc., Palo Alto, CA) was used for metabolism studies. The mobile phase included 0.1% trifluoroacetic acid in water (solvent A) and 0.1% trifluoroacetic acid in acetone (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 Sciei 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 an Avance 500 MHz NMR spectrometer (Bruker Daltonics, 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, Inc. (Wilmington, MA). Male bile duct-cannulated (BDC) F344 rats (248–275 g, 9 weeks old) were purchased from Hilltop Laboratory Animals, Inc. (Scottsdale, PA). Animals

**TABLE 1**

<table>
<thead>
<tr>
<th>Target dose of cumene in disposition studies</th>
<th>Male Rat</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose Route</strong></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Intravenous</td>
<td>1.4</td>
<td>10, 50, 100, 1000</td>
</tr>
<tr>
<td>Oral</td>
<td>1.4, 14, 140</td>
<td></td>
</tr>
</tbody>
</table>

**Materials and Methods**

**Materials.** [Ring-U-¹⁴C]cumene (specific activity 54 mCi/mmol) was obtained from Moravek Biochemicals (Brea, CA). [¹⁴C]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). Alkanulis-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 Life and Analytical Sciences (Waltham, MA). In-Flow ES scintillation cocktail was obtained from IN/US Systems (Tampa, FL). Hydrogen peroxide (H₂O₂, 30%) was purchased from Thermo Fisher Scientific (Waltham, MA). 2-Phenyl-2-propanol was purchased from Fluka (Buchs, Switzerland). 2-Phenyl-1-propanol, 2-phenyl-1,2-propanediol, 2-hydroxy-2-phenylpropionic acid, 2-phenylpropionic acid, 2-phenylpropanaldehyde, AMS, thionyl chloride, glycine, trifluoroacetic acid, dithiothreitol, heparin, β-glucuronidase from *Escherichia coli* (type VIIA, sulfatase-free), β-glucuronidase/sulfatase from *Helix pomatia* (type H-1), glucose 6-phosphate, glucose-6-phosphate dehydrogenase, and NADP⁺ were obtained from Sigma-Aldrich (St. Louis, MO). AMS oxide was purchased from TCI America (Portland, OR). The BCA Protein Assay Kit and albumin standard were purchased from Pierce Chemical (Rockford, IL).
were housed individually in all-glass metabolism cages from 1 day before 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 (National Research Council, 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 (milligrams) of unlabelled cumene to [14C]cumene was 34:1 for rats and 2808:1 for mice. 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 [14C]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 males and 10, 150, or 1000 mg/kg for females. The means ± S.D. for the concentrations and amounts of [14C]administered orally to mice 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.

**Intravenous Dosing.** Single doses were delivered by intravenous injection into the tail vein of rats and mice. The ratio (milligrams) of unlabelled cumene to [14C]cumene was 51:1 and 111:1 for rats and mice, respectively. The intravenous dose administered to rats and mice was in water-ethanol-Alkamuls-EL 620 (8:1:1, w/v/v) and administered at 1 ml/kg for rats and 4 ml/kg for mice. The target dose administered by the intravenous route to three to four male rats per treatment group was 1.4 mg/kg. The means ± S.D. for the concentrations and amounts of [14C]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 intravenous route to three mice per treatment group was 10 mg/kg. The means ± S.D. for the concentrations and amounts of [14C]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 after 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 after 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 [14C]excreted in urine. Bile was collected from the BDC rats at 0.25, 1, 2, 3, 4, 5, 6, 12, and 24 h. Triplet aliquots of urine, bile, and cage rinse were mixed with Ultima Gold scintillation cocktail and counted for [14C]content in a model 2500 TR liquid scintillation counter (LSC) (PerkinElmer Life and Analytical Sciences). The remaining samples were stored at −20°C.

For single-dose studies, feces were collected, after 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 combustion vials containing Ultima Gold scintillation cocktail and analyzed for [14C]content in the LSC.

**Blood was collected into a heparinized syringe from anesthetized animals before sacrifice. Adipose tissue (perirenal and reproductive), muscle (hind leg and trapezius), and skin (ears) were collected. To estimate the total contribution of those tissues, their total content was estimated at 0.055, 0.004, and 0.00% 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 2 N NaOH in ethanol. For the intravenous study, the tail was removed, solubilized in 2 N NaOH in ethanol, and analyzed for residual [14C]. Once dissolved, these samples were neutralized with nitric acid and bleached with H2O2 (30%). Three aliquots of these samples were weighed into scintillation vials containing Ultima Gold scintillation cocktail and analyzed for [14C] in the LSC.

**Anesthesia and Euthanasia.** At the end of all studies, animals were administered a sodium pentobarbital-based solution by intraperitoneal injection to induce surgical-level anesthesia and euthanized by exsanguination and sectioning of the diaphragm.

**Metabolite Isolation.** Metabolite isolation was performed on excrata 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 FreeZone 4.5-liter freeze dryer system (Labconco, Kansas City, MO) to remove water, or the solvents were evaporated using a SpeedVac (Thermo Fisher Scientific).

**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 to 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 performed 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 9000g for 10 min, the supernatant was removed and centrifuged at 100,000g for 1 h. The lung microsomal pellet was resuspended in 10 mM Tris, 150 mM KCl, 1 mM EDTA, 0.5 mM dithiothreitol, and 15% glycerol (pH 7.4) and 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) and then aliquoted and stored at −80°C.

**Incubations of Cumene with Microsomes and the NADPH-Regenerating System.** [14C]Cumene (1 mM, 1 μCi/sample) was incubated with microsomes (1 mg of protein/ml) in 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 were precipitated by addition of 1 ml of ice-chilled acetonitrile. After centrifugation (10,000g, 5 min), the supernatant was analyzed by HPLC.

**Synthesis of 2-Phenylpropionylglycine.** 2-Phenylpropionic acid (258 μl, 283.5 μmol) and 1.5 Eq of thionyl chloride (200 μl, 326.2 mg, 2.74

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mmol) in dichloromethane (10 ml) were stirred in an ice bath for 1.5 h. The mixture turned yellow while bubbles evolved from the solution. Triethylamine (382 µl, 2.77 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 using a SpeedVac, and the residue was analyzed by MS and ¹H NMR. The mass spectra [ES]+-MS/MS of m/z 206 [M+H]+; ESI(−)-MS/MS of m/z 162 [M−H−CO₃]−; ESI(−)-MS: m/z 208 [M+H]+; ESI(−)-MS/MS of m/z 208: m/z 190 [M+H−H₂O]−, 133 [M+H−glycine]−, and 105 [M+H−glycine−CO₃]−; and NMR spectra [¹H NMR (D₂O): 6.74–6.79 (m, 3H, phenyl-H), 3.96 (AB quartet, J = 17.5 Hz, 2H, Gly α-CH₃), 3.88 (q, J = 7.0 Hz, 1H, 2-CH), and 1.53 (d, J = 7.0 Hz, 3H, 3-CH₃)] confirmed the identity of the 2-phenylpropionylglycine.

Excretion of radioactivity as ¹⁴CO₂ in all groups surveyed was negligible in the intestines 24 h after single administration of the same dose. Male and female mice excreted ¹⁴CO₂ after single-dose administration of cumene to rats. However, in contrast to rats, little ¹⁴C remained in the intestines of mice 24 h after dosing. There was a trend toward decreased excretion in urine with increasing dose; however, because of the variance among 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 ¹⁴C as VOCs at the high dose relative to that at the lower doses (p = 0.005 and 0.002 for males and females, respectively). As in rats, excretion of ¹⁴CO₂ was negligible; however, female mice excreted less ¹⁴CO₂ (0.03 ± 0.01%) at the high dose than did males (1.6 ± 0.1%) (p = 0.0001). Females also excreted more ¹⁴C as VOCs at the high dose than did males (p = 0.02). Little or no sex-related differences in ¹⁴C excretion were observed in the range of middle doses (50–150 mg/kg) administered to mice. The pattern of ¹⁴C excretion after intravenous administration was similar to that after administration of a similar oral dose to mice. However, the amount of ¹⁴C excreted in urine was less after the intravenous dose, possibly because of the poor recovery of dose, particularly in the male mice. Repeat oral dosing had little or no effect on the excretion of ¹⁴C after daily administration of 150 mg/kg for either 3 or 7 consecutive days to female mice.

**Results**

**Excretion of Cumene-Derived Radioactivity.** The excretion data from all ¹⁴C-cumene studies are shown in Table 2. Approximately 70 to 80% of the total ¹⁴C was excreted in urine and 1% was 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 after dosing. Little ¹⁴C remained in the intestines, and excretion of dose was higher in urine 72 h after gavage administration of 14 mg/kg. In addition, the amount of ¹⁴C contained in the intestines 24 h after the last of three or seven consecutive daily doses of 14 mg/kg was significantly less than the amount observed in the intestines 24 h after single administration of the same dose. Excretion of radioactivity as ¹⁴CO₂ in all groups surveyed was negligible (data not shown). A small, but measurable quantity of ¹⁴C was excreted as VOCs after single-dose administration of cumene to rats.

**TABLE 2**

<table>
<thead>
<tr>
<th>Route</th>
<th>Frequency</th>
<th>% Dose in</th>
<th>Total Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>h</td>
<td>Urine</td>
</tr>
<tr>
<td>Male rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous</td>
<td>1.4</td>
<td>24</td>
<td>90.1 ± 5.9</td>
</tr>
<tr>
<td>Oral</td>
<td>1.4</td>
<td>24</td>
<td>77.4 ± 10.1</td>
</tr>
<tr>
<td>Oral</td>
<td>14</td>
<td>24</td>
<td>70.2 ± 3.4</td>
</tr>
<tr>
<td>Oral</td>
<td>140</td>
<td>24</td>
<td>69.8 ± 8.9</td>
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<tr>
<td>Oral</td>
<td>14</td>
<td>72</td>
<td>85.6 ± 4.0</td>
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<tr>
<td>Oral</td>
<td>14 × 3</td>
<td>24</td>
<td>73.9 ± 2.7</td>
</tr>
<tr>
<td>Oral</td>
<td>14 × 7</td>
<td>24</td>
<td>81.3 ± 5.6</td>
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<tr>
<td>Male mouse</td>
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<tr>
<td>Intravenous</td>
<td>10</td>
<td>24</td>
<td>47.4 ± 6.5</td>
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<tr>
<td>Oral</td>
<td>10</td>
<td>24</td>
<td>105.0 ± 5.0</td>
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<tr>
<td>Oral</td>
<td>50</td>
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<td>90.2 ± 6.1</td>
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<td>Oral</td>
<td>100</td>
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<td>79.3 ± 9.9</td>
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<tr>
<td>Oral</td>
<td>1000</td>
<td>24</td>
<td>80.9 ± 6.1</td>
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<td>Female mouse</td>
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<tr>
<td>Intravenous</td>
<td>10</td>
<td>24</td>
<td>74.0 ± 8.4</td>
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<td>Oral</td>
<td>10</td>
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<td>93.7 ± 3.4</td>
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<td>Oral</td>
<td>150</td>
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<td>86.5 ± 5.0</td>
</tr>
<tr>
<td>Oral</td>
<td>1000</td>
<td>24</td>
<td>79.2 ± 5.7</td>
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<tr>
<td>Oral</td>
<td>150 × 3</td>
<td>24</td>
<td>91.1 ± 11.1</td>
</tr>
<tr>
<td>Oral</td>
<td>150 × 7</td>
<td>24</td>
<td>77.2 ± 7.8</td>
</tr>
</tbody>
</table>

GL, gastrointestinal; N.D., not determined.

* Frequency of dosing; S, single dosing; R, repeated daily dosing.

* The animals were sacrificed 24 or 72 h after single dosing or 24 h after the last of three or seven consecutive daily doses.

* Stomach, small and large intestines, and their contents.

* Includes ¹⁴CO₂ collected in some studies and all surveyed tissues listed under Materials and Methods, including the digested carcasses.
Concentrations of 14C in blood, muscle, skin, and spleen were determined in tissues of these animals were in the liver, kidney, and lung, and these data, including data for blood, are presented in Table 3. The increases in the concentrations of 14C in the blood, liver, kidney, and lung were proportional to dose in the range of 1.4 to 140 mg/kg in the rat; however, the data were more variable across the range of doses in mice. The concentrations of 14C in these tissues were higher in the rat than in the mouse at comparable doses in the range of 10 to 150 mg/kg. In mice, concentrations of 14C were similar in respective tissues of males and females after gavage of 10 mg/kg; however, tissues of female mice generally contained higher amounts of 14C than those of male mice after administration of 1000 mg/kg. The tissue/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 and 150 mg/kg for rats and mice, respectively) by gavage and the tissue concentrations of 14C were determined 24 h after the last dose (Table 4). There were no significant differences in concentrations of 14C in liver, kidney, and lung in the rat after repeat dosing compared with those observed after a single dose. Concentrations of 14C in blood, muscle, skin, and spleen were significantly increased after three doses in the rat; however, only skin contained significantly elevated concentrations of 14C 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 with that after a single dose. Concentrations of 14C in blood, heart, kidney, and lung increased between three and seven doses. As shown in Fig. 2, kidney and liver contained more 14C 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/blood ratio of the tissues in all treatment groups as shown in Table 4. The high variance in concentrations in the 14C in the urinary bladder indicated that the 14C was primarily associated with residual urine rather than with the tissue itself. In the mouse, the lung and liver contained the highest mean concentrations of 14C among the blood, liver, kidney, and lung (Table 4; Fig. 2). The lung seemed to have the greatest potential among these tissues for accumulation of 14C 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 (Fig. 3A). This radiolabeled peak at 39.8 min had a strong UV absorption at 254 nm (Fig. 3B). The metabolite at 39.8 min was suspected to be AMS because of the strong UV absorption at 254 nm and showed a polarity similar to that of cumene. HPLC analysis of authentic AMS demonstrated a retention time similar to 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 (Fig. 3, A and B), 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 AMS, and therefore the VOCs was not analyzed.

**Urinary Metabolites.** Seventy percent and greater 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 exposed with high oral doses revealed a number of radiolabeled peaks designated as M1 to M16 (Fig. 4, A–C). Some of the urine samples were subjected to hydrolysis by glucuronidase/sulfatase from *H. pomatia* or glucuroni- dase or sulfatase and did not ionize upon MS analysis. A representative HPLC radiochromatogram from hydrolysis of male rat urine is shown in Fig. 4D. Cumene was not detected in urine (data not shown). The urinary metabolites were characterized by MS and/or 1H NMR analysis (supplemental data). The structures of the metabolites identified are shown in Fig. 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 reanalysis 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, probably the dehydration products. The structure of M1 remains unknown, but the data suggest that it is probably 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 (Fig. 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] +
### TABLE 4

**Cumene-derived radioactivity in all surveyed tissues 24 h after single or repeat dosing (consecutive daily dosing) of 14 mg/kg to male rats and 150 mg/kg to female mice**

Each value represents the mean ± S.D. of four animals per treatment group.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Male Rat</th>
<th></th>
<th></th>
<th>Female Mouse</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Dose</td>
<td>3 Doses</td>
<td>7 Doses</td>
<td>1 Dose</td>
<td>3 Doses</td>
<td>7 Doses</td>
</tr>
<tr>
<td></td>
<td>Conc. T/B</td>
<td>Conc. T/B</td>
<td>Conc. T/B</td>
<td>Conc. T/B</td>
<td>Conc. T/B</td>
<td>Conc. T/B</td>
</tr>
<tr>
<td></td>
<td>mmol-Eq/g</td>
<td>mmol-Eq/g</td>
<td>mmol-Eq/g</td>
<td>mmol-Eq/g</td>
<td>mmol-Eq/g</td>
<td>mmol-Eq/g</td>
</tr>
<tr>
<td>Adipose</td>
<td>12 ± 7</td>
<td>3.1 ± 1.7</td>
<td>4.7 ± 2.0</td>
<td>1.4 ± 0.5</td>
<td>0.89 ± 0.56</td>
<td>0.97 ± 0.56</td>
</tr>
<tr>
<td>Urinary Bladder</td>
<td>72 ± 38</td>
<td>19 ± 12</td>
<td>24 ± 16</td>
<td>106 ± 21</td>
<td>14 ± 10</td>
<td>21 ± 1.1</td>
</tr>
<tr>
<td>Blood</td>
<td>3.8 ± 0.3</td>
<td>8.1 ± 0.4</td>
<td>6.3 ± 0.6</td>
<td>3.6 ± 1.0</td>
<td>11 ± 10</td>
<td>21 ± 1.0</td>
</tr>
<tr>
<td>Brain</td>
<td>1.4 ± 0.2</td>
<td>3.2 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>1.1 ± 0.4</td>
<td>0.65 ± 0.22</td>
<td>0.41 ± 0.05</td>
</tr>
<tr>
<td>Heart</td>
<td>2.4 ± 0.3</td>
<td>6.0 ± 1.2</td>
<td>4.1 ± 0.1</td>
<td>1.4 ± 0.6</td>
<td>0.63 ± 0.03</td>
<td>0.53 ± 0.09</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.2 ± 2</td>
<td>5.9 ± 1.3</td>
<td>3.2 ± 0.1</td>
<td>0.6 ± 0.3</td>
<td>2.9 ± 0.3</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Liver</td>
<td>22 ± 3</td>
<td>5.9 ± 1.4</td>
<td>3.2 ± 0.1</td>
<td>7.0 ± 0.6</td>
<td>4.7 ± 0.1</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>Lung</td>
<td>6.1 ± 2.6</td>
<td>1.6 ± 0.6</td>
<td>1.1 ± 0.1</td>
<td>1.9 ± 0.4</td>
<td>3.2 ± 0.1</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.0 ± 0.5</td>
<td>0.27 ± 0.13</td>
<td>0.49 ± 0.07</td>
<td>1.9 ± 0.4</td>
<td>0.29 ± 0.16</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>Skin</td>
<td>2.5 ± 0.5</td>
<td>0.66 ± 0.17</td>
<td>1.0 ± 0.3</td>
<td>7.3 ± 2.9</td>
<td>1.2 ± 0.5</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.4 ± 0.8</td>
<td>0.62 ± 0.17</td>
<td>0.94 ± 0.23</td>
<td>5.7 ± 3.2</td>
<td>0.91 ± 0.35</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.0 ± 0.5</td>
<td>0.1 ± 0.1</td>
<td>0.49 ± 0.61</td>
<td>0.29 ± 0.9</td>
<td>0.7 ± 0.04</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Testes</td>
<td>1.3 ± 0.4</td>
<td>0.33 ± 0.12</td>
<td>0.28 ± 0.05</td>
<td>1.3 ± 0.0</td>
<td>0.78 ± 0.21</td>
<td>0.90 ± 0.54</td>
</tr>
</tbody>
</table>

*Statistically higher (p < 0.05) in the same tissues from three or seven dose studies versus the single-dose study.

#Statistically higher (p < 0.05) in the same tissues from the seven-dose study versus the three-dose study.

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DISPOSITION AND METABOLISM OF CUMENE IN MICE
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), 5.50 (s, 1H, olefin H), and 2.20 (s, 3H, 3-CH₃). The HPLC reanalysis 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 (Fig. 4D). This metabolite did not ionize upon ESI-MS analysis. HPLC reanalysis 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, probably the dehydration products. The structure of M₄ remains unknown, but the data suggest that it is probably 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 dihydroxy-cumene 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 reanalysis showed that it had decomposed to a peak with a retention time similar to that of 2-phenyl-1,2-propandiol (17.6 min). When the metabolite was isolated by HPLC UV cell, it was stable for a ¹H NMR analysis. All five phenyl protons were observed by NMR, indicating that there was no ring oxidation. The presence of 3-CH₃ as a singlet and 1-CH₂ 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-propandiol was as follows: δ 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-CH₂), and 1.57 (s, 3H, 3-CH₃). Because of sulfate conjugation, the 1-CH₂ AB quartet in M₅ (δ 4.17) was more downfield compared with that in the hydrolysis product (δ 3.76). The position of sulfate conjugation was probably at the 1-position, which was less hindered. This metabolite was tentatively identified as 2-hydroxy-2-phenylpropylsulfate (M₅).

MS analysis of M₆ with an HPLC retention time of 16.6 min gave a molecular weight of 328, equivalent to cumene (120) + 2 [O] + glucuronic acid (176). The NMR spectrum of M₆ 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-CH₃ as a singlet and 1-CH₂ as an AB quartet indicated that hydroxylation occurred at the 1- and 2-positions of the isopropyl side chain. The spectral data of M₆ were consistent with formation of a 2-phenyl-1,2-propanediol glucuronide. The position of glucuronide conjugation was probably at the 2-position, as the steric effect might have resulted in 3-CH₃ and 1-CH₂ being further downfield in

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**Fig. 3.** Representative HPLC radiochromatogram (A) and UV (254 nm) chromatogram (B) of cumene and AMS in the expired air (0–6 h) of a male mouse dosed orally with a single 1000 mg/kg dose.

**Fig. 4.** Representative HPLC radiochromatograms of cumene metabolites (M₁–M₁₆) in urine (0–24 h) of a male rat dosed orally with a single 140 mg/kg dose (A), urine (0–24 h) of a female mouse dosed orally with a single 1000 mg/kg dose (B), urine (0–24 h) of a male mouse dosed orally with a single 1000 mg/kg dose (C), glucuronidase/sulfatase-hydrolyzed urine (0–24 h) of a male rat dosed orally with a single 140 mg/kg dose (D), and bile (0–6 h) of a BDC male rat dosed intravenously with a 1.4 mg/kg single dose (E).
M6 than in its isomer, M7. This metabolite was identified as 2-phenyl-1,2-propandiol 2-glucuronide (M6).

M7 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 M7 showed five phenyl protons, indicating no ring oxidation. The presence of 3-CH₃ as a singlet and 1-CH₂ 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) as that of the parent glucuronide and of the 2-phenyl-1,2-propandiol authentic standard. Epimerization of glucuronide 1-H took place during isolation, so the signals attributed to the 1-glucuronide were also observed by NMR (data not shown). All data suggested formation of 2-phenyl-1,2-propandiol monoglucuronide. Because M7 was more abundant than its isomer M6, the position of glucuronide conjugation was probably at the less hindered 1-position. A previous metabolism study showed formation of 2-phenyl-1,2-propandiol 1-glucuronide as a major metabolite of AMS (De Costa et al., 2001).

M8 had an HPLC retention time (20.3 min) similar to that of authentic 2-hydroxy-2-phenylpropionic acid. MS analysis showed that this metabolite 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 and 2-phenylpropionic acid (M16). Intraperitoneal administration of 2-phenylpropionic acid to rats showed that 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 probably 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). 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-phenylpropanol anion (m/z 167) and 2-acycetaminocarboxylic acid (m/z 128) was consistent with an N-acetylcysteine attached at the β-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 α-carbon (a tertiary carbon). The metabolite was probably S-(2-hydroxy-2-phenylpropyl)-N-acetylcysteine, a metabolite identified in the urine of male rats dosed with AMS (De Costa et al., 2001). In general, M12 was more abundant in rat urine, and only a trace amount was observed in mouse urine. M12 was tentatively identified as S-(2-hydroxy-2-phenylpropyl)-N-acetylcysteine.

MS analysis showed that M13 had a molecular weight of 312, consistent with formation of a monoxygenated cumene glucuronide [cumene (120) + O + glucuronic acid (176)]. β-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. M13 was tentatively identified as 2-phenyl-1-propanol glucuronide.

Three minor metabolites with retention times of 27.6, 28.4, and 29.5 min were occasionally observed in rat or mouse urine. These metabolites had retention times identical to those of authentic 2-phenyl-2-propanol (27.6 min, M14), 2-phenyl-1-propanol (28.4 min, M15), and 2-phenylpropionic acid (29.5 min, M16).

Most of these metabolites (M1–M16) were also detected in urine from mice treated with lower doses of cumene. The percent dose of M1 to M16 in male rat urine from all treatment groups is shown in Table 5. M9 and M10 coeluted and were counted together. β-Glucuronidase hydrolysis revealed that M10 was only a minor metabolite (Fig. 4D); therefore, M9 was the most abundant metabolite in rat urine (38–50% of all radiolabeled peaks). M7 and M8 each constituted 11 to 20% of all radiolabeled peaks. The percentage of all other metabolites in male rat urine was less than 10% except for M3, 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 of cumene, 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 are shown in Table 6. M9 accounted for 30 to 43% of all radiolabeled peaks in mouse urine. M8 constituted 11 to 20% of all radiolabeled peaks. The percentage of M7 and M5 ranged from 6 to 17% and from 3 to 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 intravenous 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 to 6 h was analyzed by HPLC to reveal several radiolabeled peaks (Fig. 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 (Fig. 6). Female mouse lung microsomes (Fig. 6A) metabolized more cumene than female mouse liver microsomes (Fig. 6B), female rat lung microsomes (Fig. 6C), or female rat liver microsomes (Fig. 6D). The percentage of these cumene metabolites in microsomal incubations is shown in Table 7.

**Discussion**

The present study demonstrated that cumene was absorbed after oral administration to male rats and mice of both sexes and excreted primarily in urine. The excretion of 14C as VOCs was dependent on dose, sex, species, and route of administration. Expiration of 14C VOCs increased at the high doses, especially in mice, implying saturation of specific metabolic pathways. Female mice excreted more 14C as VOCs and CO2 (p = 0.05) and retained more 14C in tissues (p = 0.0001) than did males at the high dose, indicating that male mice metabolized cumene more efficiently.
than females. More $^{14}$C was excreted as VOCs after an intravenous dose versus the comparable oral dose ($p = 0.002$).

The substantial amount of $^{14}$C in the intestines 24 h after intravenous injection in rats suggested biliary excretion of cumene and/or its metabolites. This result was confirmed in BDC rats after excretion of 37% of a total cumene dose in bile within 24 h after 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 versus 10 mg/kg male and female mouse oral dosing). The $^{14}$C concentrations in the kidneys of male rats were much higher than those in mice at comparable doses ($p < 0.0001$ for 14 mg/kg rat versus 10 mg/kg male and female mouse oral dosing) and may indicate binding of cumene and/or metabolites to male rat-specific $\alpha_{2a}$-globulin in the kidneys (Strasser 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 (National Toxicology Program, 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 (National Toxicology Program, 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 Fig. 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)sulfate (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
rulled 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 than in female mice, 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 and 1000 mg/kg, respectively) indicated that AMS accounted for 3 to 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 after inhalation exposure (National Toxicology Program, 2009). Because female mice were more susceptible, cumene-metabolizing activity was studied in female mouse lung and liver microsomes and compared with that in male rat lung and liver microsomes. The results are shown in Fig. 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 that 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 National Toxicology Program toxicity studies. In the in vitro microsomal incubation study demonstrated that lung microsomes metabolized 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 14C in mouse lung after multiple doses of [14C]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 severalfold 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 (Buckpitt et al., 1995; Hynes et al., 1999). Furthermore, 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 isofrom (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 α2u-globulins, can lead to granular casts and single cell necrosis, increased cell division and tubule hyperplasia, and finally renal tubule adenoma and carcinoma (Rodgers and Baetcke, 1993). If the above proposed tumorigenicity pathway is correct, it follows that cumene would not be considered a renal tumor risk in humans because α2u-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 and Caudill, 1992; Lehman-McKeeman, 1993; Swenberg, 1993).

Other possible reactive metabolites include the aren 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 (Fig. 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 support enterohepatic circulation of cumene and/or its metabolites. In addition, 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 seven consecutive daily doses, which correlates with the higher incidence of alveolar/bronchiolar adenoma and carcinoma observed in lungs of cumene-treated mice (National Toxicology Program, 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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Dean A. Kracko helped with MS analysis. Vicki Fisher helped in assembly of the manuscript. Dr. Karen Ann Smith (University of New Mexico) assisted with the NMR experiments.

Authorship Contributions

Participated in research design: Chen, McDonald, Dix, and Sanders.

Conducted experiments: Chen, Kramer, Thomas, and McDonald.
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Disposition and Metabolism of Cumene in F344 Rats and B6C3F1 Mice

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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 6-H), 1.54 (s, 6H, 1,3-CH3). Hydrolyzed by sulfatase. ESI(−)-MS: m/z 231 [M - H]−, 213 [M - H - H2O]−, 151 [M - H - SO3]−.</td>
</tr>
<tr>
<td>M4</td>
<td>Not identified</td>
<td>14.4</td>
<td></td>
</tr>
<tr>
<td>----</td>
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</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.

<table>
<thead>
<tr>
<th>M5</th>
<th>2-hydroxy-2-phenylpropylsulfate</th>
<th>15.2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

$^1$H NMR (D₂O): $\delta$ 7.53 (d, J = 8.5 Hz, 2H, phenyl 2,6-H), 7.46 (t, J = 8.5 Hz, 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.

<table>
<thead>
<tr>
<th>M6</th>
<th>2-phenyl-1,2-propanediol 2-glucuronide</th>
<th>16.6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

$^1$H NMR (D₂O): $\delta$ 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.

<table>
<thead>
<tr>
<th>M7</th>
<th>2-phenyl-1,2-propanediol 1-glucuronide</th>
<th>17.6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

$^1$H NMR (D₂O): $\delta$ 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.

<table>
<thead>
<tr>
<th>M8</th>
<th>2-phenyl-2-hydroxypropionic acid</th>
<th>20.3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$H NMR (D₂O): $\delta$ 7.59 (d, J = 7.5 Hz, 2H, phenyl-2,6-H), 7.48 (t, J = 9.0 Hz, 2H, phenyl-2,6-H), 7.43 (t, J = 9.0 Hz, 2H, phenyl-3,5-H), 7.36 (t, J = 7.5 Hz, 1H, phenyl-4-H), 4.50 (d, J = 7.5 Hz, 1H, Gluc 1’-H), 4.20 (d, J = 11.0 Hz, 1H, Gluc 5’-H), 3.89 (AB quartet, J = 11.5 Hz, 2H, 1-CH₂), 3.57-3.47 (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.


ESI(-)-MS/MS of m/z 327: m/z 193 [glucuronide]⁻, 151 [M - H - glucuronide]⁻.


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].

$^1$H 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].

$^1$H 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></th>
<th>Substance</th>
<th>RRT</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>M14</td>
<td>2-phenyl-2-propanol</td>
<td>27.6</td>
<td>Comparison with authentic 2-phenyl-2-propanol.</td>
</tr>
<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>
