Disposition and Metabolism of Cumene in F344 Rats and B6C3F1 Mice

Ling-Jen Chen, Christopher J. Wegerski, Daniel J. Kramer, Leslie A. Thomas, Jacob D. McDonald, Kelly J. Dix, and J. Michael Sanders

Lovelace Respiratory Research Institute, Albuquerque, New Mexico (L.-J.C., C.J.W., D.J.K., L.A.T., J.D.M., K.J.D.); and National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina (J.M.S.)

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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 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 (Budavari, 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 burned 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 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.

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 that was lethal in some of the animals (U.S. Environmental Protection Agency, 1988). Subsequently, there is human exposure to cumene

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ABBREVIATIONS: AMS, α-methylstyrene; HPLC, high-performance liquid chromatography; ESI, electrospray ionization; MS, mass spectrometry; MS/MS, tandem mass spectrometry; BDC, bile duct-cannulated; LSC, liquid scintillation counter; VOC, volatile organic compound.

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Metabolism in female rat lung and liver microsomes was also studied for comparison.

Materials and Methods

Materials. [Ring-U-$^{14}$C]Cumene (specific activity 54 mCi/mmol) was obtained from Moravek Biochemicals (Breca, CA). [$^{14}$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 Acrors Organics (Fair Lawn, NJ). Alkanuls-EL 620 (>99% castor oil ethoxylates) was obtained from Rhodia (Crabury, 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 (H$_2$O$_2$, 30%) was purchased from Thermo Fisher Scientific (Waltham, MA). 2-Phenyl-1-propanol, 2-phenyl-1,2-propanediol, and 2-hydroxy-2-phenylpropionic acid were purchased from Fluka (Buchs, Switzerland). 2-Phenyl-2-propanol was purchased 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 Bio-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, 150 mm; Phenomenex, Torrance, CA) was used for disoposition studies. The mobile phase included H$_2$O (solvent A) and acetoni- trile (solvent B). The elution began with a linear gradient from 10% 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, 250 mm; Varian, Inc., Palo Alto, CA) was used for metabolism studies. The mobile phase included 0.1% trifluoro-aic acid in water (solvent A) and 0.1% trifluoroacetic acid in acetoni- trile (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.

$^1$H NMR spectra were acquired on a Avance 500 MHz NMR spectrometer (Bruker Daltronics, Billerica, MA). The chemical shifts are reported in parts per million relative to D$_2$O (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

<p>| TABLE 1 |</p>
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<th>Target dose of cumene in disposition studies</th>
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<tr>
<td><strong>Dose Route</strong></td>
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<td><strong>mg/kg</strong></td>
</tr>
<tr>
<td>Intra venous</td>
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<tr>
<td>Oral</td>
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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 administered by gavage. All oral doses were in corn oil and administered at 5 ml/kg to rats and 10 ml/kg to mice. The ratio (milligrams) of unlabeled cumene to \( ^{14} \text{C} \) cumene was 3:1 for rats and 280:1 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 \( ^{14} \text{C} \) administered to rats were 1.7 ± 0.3 mg (92 ± 14 \( \mu \text{Ci} \)/kg), 18 ± 1 mg (90 ± 4 \( \mu \text{Ci} \)/kg), or 149 ± 10 mg (102 ± 7 \( \mu \text{Ci} \)/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 \( ^{14} \text{C} \) administered orally to mice were 13 ± 0 mg (1129 ± 20 \( \mu \text{Ci} \)/kg), 53 ± 1 mg (1191 ± 0 \( \mu \text{Ci} \)/kg), 99 ± 1 mg (1127 ± 6 \( \mu \text{Ci} \)/kg), or 1071 ± 46 mg (1265 ± 54 \( \mu \text{Ci} \)/kg) to males and 11 ± 0 mg (1129 ± 6 \( \mu \text{Ci} \)/kg), 151 ± 1 mg (1133 ± 7 \( \mu \text{Ci} \)/kg), and 1064 ± 59 mg (1504 ± 84 \( \mu \text{Ci} \)/kg) to females.

**Intravenous Dosing.** Single doses were delivered by intravenous injection into the tail vein of rats and mice. The ratio (milligrams) of unlabeled cumene to \( ^{14} \text{C} \) 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, v/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 \( ^{14} \text{C} \) administered to rats were 1.1 ± 0.0 mg (55 ± 2 \( \mu \text{Ci} \)/kg) and 2.1 ± 0.1 mg (43 ± 2 \( \mu \text{Ci} \)/kg) to nonanunnulated 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 \( ^{14} \text{C} \) administered to mice were 7 ± 0 mg (5 ± 0 \( \mu \text{Ci} \)/kg) for males and 6 ± 2 mg (4 ± 1 \( \mu \text{Ci} \)/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 \( ^{14} \text{C} \) 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 in the LSC for determination of \( ^{14} \text{C} \) content. Once dissolved, these samples were neutralized with nitric acid and bleached with \( \text{H}_2\text{O}_2 \) (30%). Triplicate aliquots of these samples were weighed into scintillation vials containing Ultima Gold scintillation cocktail and analyzed for \( ^{14} \text{C} \) 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 excreta collected within 24 h of dosing. Metabolites were isolated from HPLC by collecting the radiolabeled peaks detected with a β-ARAM solid cell or by collecting the UV-absorbing peaks with the detection at 254 nm. The collected samples were placed under a stream of \( \text{N}_2 \) to remove acetonilide 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 ~76 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.** \( ^{14} \text{C} \) 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 acetoniolinine solution (type VII, ~2000 U, sulfatase-free) or β-glucuronidase/sulfatase from \( H. \) pomatia (β-glucuronidase ~2000 U and sulfatase ~76 U) in a 0.1 M sodium acetate buffer (pH 6.8 for the enzyme from \( E. \) coli and pH 5.0 for the enzyme 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.

**Synthesis of 2-Phenylpropionylglycine.** 2-Phenylpropionic acid (258 μl, 283.5 mg, 1.83 mmol) and 1.5 Eq of thionyl chloride (200 μl, 326.2 mg, 2.74
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, 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 using a SpeedVac, and the residue was analyzed by MS and H NMR. The mass spectra [ESI(−)-MS/MS] of 206 [M + H]−; ESI(−)-MS/MS of m/z 162 [M − H − CO₂]; ESI(+)-MS/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 − CO₂]+; and NMR spectra [1H NMR (D₂O): 67.46–7.39 (m, 5H, phenyl-H), 3.96 (AB quartet, J = 7.46–7.39 (m, 5H, phenyl-H), 3.96 (AB quartet, J = 7.46–7.39 (m, 5H, phenyl-H), 3.96 (AB quartet, J = 7.46–7.39 (m, 5H, phenyl-H)] confirmed the identity of the 2-phenylpropionylglycine. Excretion of radioactivity as 14CO₂ in all groups surveyed was negatively correlated with dose. In general, the amount of 14CO₂ excreted was higher in urine 72 h after gavage administration of a similar oral dose to mice. However, the amount of 14C in excreta of a similar oral dose to mice. However, the amount of 14C in excreta 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 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 14C (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.

### Statistical Analysis

Statistical analysis of data was performed using the GraphPad Prism (GraphPad Software Inc., San Diego, CA) F test and two-tailed t tests. Values were considered significantly different at p ≤ 0.05.

## 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>
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<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>mg/kg</td>
<td>h</td>
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<tr>
<td></td>
<td></td>
<td>Urine</td>
<td>Feces</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Male rat</td>
<td>Intravenous</td>
<td>1.4</td>
<td>90.1 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>1.4</td>
<td>77.4 ± 10.1</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>14</td>
<td>70.2 ± 3.4</td>
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<tr>
<td></td>
<td>Oral</td>
<td>140</td>
<td>69.8 ± 8.9</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>14</td>
<td>85.6 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>14 × 3</td>
<td>73.9 ± 2.7</td>
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<tr>
<td></td>
<td>Oral</td>
<td>14 × 7</td>
<td>81.3 ± 5.6</td>
</tr>
<tr>
<td>Male mouse</td>
<td>Intravenous</td>
<td>S 10</td>
<td>47.4 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>10</td>
<td>105.0 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>50</td>
<td>90.2 ± 6.1</td>
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<tr>
<td></td>
<td>Oral</td>
<td>100</td>
<td>79.3 ± 9.9</td>
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<tr>
<td></td>
<td>Oral</td>
<td>1000</td>
<td>80.9 ± 6.1</td>
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<tr>
<td>Female mouse</td>
<td>Intravenous</td>
<td>10</td>
<td>74.0 ± 8.4</td>
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<td></td>
<td>Oral</td>
<td>10</td>
<td>93.7 ± 3.4</td>
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<td></td>
<td>Oral</td>
<td>150</td>
<td>86.5 ± 5.0</td>
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<td></td>
<td>Oral</td>
<td>1000</td>
<td>79.2 ± 5.7</td>
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<tr>
<td></td>
<td>Oral</td>
<td>150 × 3</td>
<td>91.1 ± 11.1</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>150 × 7</td>
<td>77.2 ± 7.8</td>
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Gl, gastrointestinal; N.D., not determined.

a Frequency of dosing: S, single dosing; R, repeated daily dosing.

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

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

d Includes 14C collected in some studies and all surveyed tissues listed under Materials and Methods, including the digested carcasses.
tected 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 $^{14}$C 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 $^{14}$C 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 $^{14}$C 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 $^{14}$C 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 $^{14}$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 after repeat dosing compared with 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 with that 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 with that after a single dose. Concentrations of $^{14}$C in blood, heart, kidney, and lung increased between three and seven doses. As shown in Fig. 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/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 with 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; Fig. 2). The lung seemed 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 (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 $^{14}$C as VOCs, 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 dosed 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 glucuronidase from *E. coli* (sulfatase-free) to recognize glucuronide or sulfate conjugates. 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 $^1$H 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 and may be a dihydrodiol, which can dehydrate to form a 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 $^{14}$C as VOCs, and therefore the VOCs was not analyzed.

<table>
<thead>
<tr>
<th>Route</th>
<th>Dose</th>
<th>Time</th>
<th>Blood Conc.</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lung</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>h</td>
<td>nmol-Eq/g</td>
<td>T/B</td>
<td>nmol-Eq/g</td>
<td>T/B</td>
</tr>
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<td>Male rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous</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>
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<td>0.37 ± 0.10</td>
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<td>2.8 ± 1.2</td>
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<td>22 ± 3</td>
<td>5.9 ± 1.4</td>
<td>33 ± 3</td>
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<td>Oral</td>
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<td>35 ± 6.5</td>
<td>146 ± 38</td>
<td>4.1 ± 0.6</td>
<td>279 ± 30</td>
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<td>Oral</td>
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<td>72</td>
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<td>1.1 ± 0.3</td>
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<td></td>
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<td>Intravenous</td>
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<td>0.67 ± 0.11</td>
<td>5.2 ± 0.5</td>
<td>0.66 ± 0.03</td>
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<td>7.8 ± 2.6</td>
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<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>
</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>
</tr>
<tr>
<td>Female mouse</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous</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>
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<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>
</tr>
<tr>
<td>Oral</td>
<td>150</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>
</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>
</tr>
</tbody>
</table>

T/B, tissue/blood ratio.
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

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 Dose</th>
<th>3 Doses</th>
<th>7 Doses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. T/B</td>
<td>Conc. T/B</td>
<td>Conc. T/B</td>
</tr>
<tr>
<td>Male Rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipose</td>
<td>1.2 ± 7</td>
<td>3.1 ± 1.7</td>
<td>2.6 ± 2.0</td>
</tr>
<tr>
<td>Blood</td>
<td>3.8 ± 0.3</td>
<td>5.8 ± 1.1*</td>
<td>6.3 ± 2.7</td>
</tr>
<tr>
<td>Brain</td>
<td>1.4 ± 0.2</td>
<td>3.2 ± 1.2*</td>
<td>2.1 ± 1.0</td>
</tr>
<tr>
<td>Heart</td>
<td>2.4 ± 0.3</td>
<td>4.0 ± 1.2*</td>
<td>4.0 ± 1.6*</td>
</tr>
<tr>
<td>Kidney</td>
<td>32.3 ± 0</td>
<td>60.4 ± 2.7</td>
<td>57.5 ± 9.5</td>
</tr>
<tr>
<td>Liver</td>
<td>22.3 ± 3</td>
<td>31.9 ± 5.4</td>
<td>32.3 ± 5.1</td>
</tr>
<tr>
<td>Lung</td>
<td>6.1 ± 2.6</td>
<td>6.6 ± 1.8</td>
<td>7.4 ± 2.3*</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.0 ± 0.5</td>
<td>2.8 ± 0.1*</td>
<td>1.9 ± 1.4*</td>
</tr>
<tr>
<td>Skin</td>
<td>2.5 ± 0.5</td>
<td>5.8 ± 1.8*</td>
<td>7.3 ± 2.9*</td>
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<tr>
<td>Spleen</td>
<td>2.4 ± 0.8</td>
<td>5.4 ± 1.4*</td>
<td>5.7 ± 3.2</td>
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<tr>
<td>Thyroid</td>
<td>0.2 ± 2.5</td>
<td>3.8 ± 1.9</td>
<td>2.9 ± 2.9*</td>
</tr>
<tr>
<td>Testes</td>
<td>1.3 ± 0.4</td>
<td>9.1 ± 7.0</td>
<td>1.8 ± 1.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Female Mouse</th>
<th>1 Dose</th>
<th>3 Doses</th>
<th>7 Doses</th>
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<td></td>
<td>Conc. T/B</td>
<td>Conc. T/B</td>
<td>Conc. T/B</td>
</tr>
<tr>
<td>1 Dose</td>
<td>1.4 ± 0.5</td>
<td>0.9 ± 0.5</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>3 Doses</td>
<td>1.8 ± 0.5</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>7 Doses</td>
<td>5.7 ± 4.2</td>
<td>5.7 ± 0.7*</td>
<td>5.7 ± 0.7*</td>
</tr>
</tbody>
</table>

S.D. of four animals per treatment group.

* 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.

Note: ND: Not detected; T/B: Tissue/blood ratio.
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 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 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 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 spectral data of M5 were consistent with formation of a 2-phenyl-1,2-propandiol monoglucuronide. 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

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).

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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 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 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 3-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. Because of 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 coeluted 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 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 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-phenylpropanoic acid (m/z 167) and 2-acetylaminoacrylate (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-phenylpropionyl)-N-acetylcysteine, a metabolite identified in the urine of male rats dosed with AMS (De Costa et al., 2001). In general, acetylcysteine, a metabolite identified in the urine of male rats (Fig. 4D); therefore, uronidase hydrolysis revealed that which exception was in urine of male rats dosed orally with 1.5 mg/kg in HPLC analyses and, therefore, were quantified together. The only N-phenylpropyl)-acetylcysteine.

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-1-propanol. M13 was tentatively identified as 2-phenyl-1-propanol glucuronide.

Most of these metabolites (M1–M16) were also detected in urine from animals 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 in which 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 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.

**TABLE 5**

Percentage of dose of cumene metabolites in cumulative 0–24 h male rat urine and 0–6 h male rat bile

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Normal Male Rat Urine</th>
<th>BDC Male Rat Bile (1.4 mg/kg i.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>140 mg/kg p.o.</td>
<td>14 mg/kg p.o.</td>
</tr>
<tr>
<td>M1</td>
<td>N.D</td>
<td>N.D</td>
</tr>
<tr>
<td>M2</td>
<td>Trace</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>N.D</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&lt;sup&gt;e&lt;/sup&gt;</td>
<td>38.1 ± 2.2</td>
<td>47.0 ± 1.3</td>
</tr>
<tr>
<td>M11</td>
<td>N.D</td>
<td>N.D</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&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Trace</td>
</tr>
<tr>
<td>M15</td>
<td>N.D</td>
<td>N.D</td>
</tr>
<tr>
<td>M16</td>
<td>2.1 ± 0.5</td>
<td>Trace</td>
</tr>
</tbody>
</table>

N.D., not detected.
<sup>a</sup>A trace amount was observed but not quantified.
<sup>b</sup>n = 2.
<sup>c</sup>n = 3.
<sup>d</sup>M10 is a minor metabolite coeluted with M9.
<sup>e</sup>n = 1.
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 $\alpha_2$-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)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 $S$-($\pm$)-2-phenylpropionic acid was not from oxidation of $R$-($\pm$)-2-phenyl-1-propanol (Ishida and Matsumoto, 1985); therefore, formation of M16 from this pathway is highly feasible. However, $R$-($\pm$)-2-phenylpropionic acid has been shown to partially isomerize to its $S$-($\pm$)-isomer in rats (Yamaguchi and Nakamura, 1985); therefore, formation of M16 from oxidation of M14 cannot be

![Fig. 6. Representative HPLC radiochromatograms of cumene and metabolites in female mouse lung (A), female mouse liver (B), female rat lung (C), and female rat liver microsomal incubations (D) (100 µl each).](image-url)
ruling out. 2-Phenylpropionic acid (M16) was further metabolized to a glucuronide conjugate (M10), predominantly in rats, and a glyoxal 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 female 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 (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/bronchial adenoma and carcinoma observed in the lung of cumene-treated mice in the National Toxicology Program 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 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 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/bronchial 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 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 (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-globulins, can lead to granular 2u-globulins, can lead to granular hyaline droplets, which contain 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/bronchial 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.

<table>
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<th>Metabolite</th>
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<td>Liver Microsomes</td>
<td>Lung Microsomes</td>
<td>Liver Microsomes</td>
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<td>0.7 ± 0.9*</td>
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<td>Cumene</td>
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<td>81.2 ± 3.4</td>
<td>84.3 ± 3.1</td>
<td>91.0 ± 0.7</td>
</tr>
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N.D., not detected.

* AMS was detected in two (1.8 and 1.1%) of four incubations.
REFERENCES


Wolfe MA, Rowe VK, McCollister DD, Hollingsworth RL, and Owen F (1956) Toxicological studies of certain alkylated benzenes and benzene; experiments on laboratory animals. AMA Arch Ind Health 14:387–398.


Address correspondence to: Dr. Jacob D. McDonald, Lovelace Respiratory Research Institute, 2425 Ridgecrest Dr. SE, Albuquerque, NM 87108. E-mail: jmcdonal@lrr.org

Performed data analysis: Chen and Wegerski.
Wrote or contributed to the writing of the manuscript: Chen, Wegerski, and Sanders.