Disposition and Metabolism of Cumene in F344 Rats and B6C3F1 Mice^[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 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 (Seńczuk and Litewka, 1976). Short-term exposure in humans has been reported to cause dizziness, drowsiness, slight incoordination, unconsciousness, and irritation to the eyes, skin, and respiratory tract (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 *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, <http://www.epa.gov/iris>; Hazardous Substances Data Bank online database, <http://www.toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB, 2005>). However, when exposed to cumene vapor, a higher incidence of alveolar/bronchiolar adenoma and carcinoma was observed in the lungs of mice of both sexes but not in rats (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-

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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.

Chemical Formula: C₉H₁₂

Molecular Weight: 120.2

* indicates position of ¹⁴C label

FIG. 1. Chemical structure, formula, and molecular weight of [¹⁴C]cumene.

tional Toxicology Program, 2007b), and benzofuran (National Toxicology Program, 1989), which are all structurally related. In contrast, a higher incidence of adenoma and carcinoma was observed in the kidney and not in the lung of male rats (and not female rats) versus controls in the 2-year toxicology and carcinogenesis studies conducted by the National Toxicology Program (2009). The LC₅₀ is 2000 ppm in mice that were exposed to cumene vapor for 7 h, and this exposure has been shown to cause various degrees of toxicity to the liver, spleen, and kidneys (Werner et al., 1944). Increases in organ weights are the most prominent effects in both male and female rats exposed to 500 or 1200 ppm of cumene vapor for 6 h/day, 5 days/week for 13 weeks (Cushman et al., 1995).

The oral LD₅₀ values of cumene in rats and mice are 1400 and 12,750 mg/kg, respectively (Wolf et al., 1956; Centers for Disease Control and Prevention, 1996, <http://www.cdc.gov/niosh/idlh/98828.html>). An increase in kidney weight was observed in female rats exposed to daily 462 or 769 mg/kg cumene 5 days/week for 6 months by the oral route (Wolf et al., 1956).

Previous metabolism studies of cumene in rats have found 2-phenyl-2-propanol (**M14**) to be the major metabolite. Administration of radiolabeled cumene to F344 rats via intravenous injection, inhalation, or oral gavage demonstrated that greater than 70% of the doses were excreted in urine regardless of the route of administration, and **M14** and its glucuronide or sulfate conjugates accounted for >50% of urinary excretion (U.S. Environmental Protection Agency, <http://www.epa.gov/iris>).

To the best of our knowledge, there are no reported absorption, distribution, metabolism, and excretion studies of cumene in mice. In addition, species and/or sex differences in the fate of cumene may account for the differential carcinogenic response observed in rats and mice in the 2-year National Toxicology Program toxicity studies of cumene. The present studies were designed to investigate the absorption, distribution, metabolism, and excretion of single or consecutive daily doses of [¹⁴C]cumene administered orally to male F344 rats or male and female B6C3F1 mice at doses ranging from 1.4 to 1000 mg/kg (Table 1). The doses were based on the oral LD₅₀ of cumene, which was approximately 10-fold higher in mice than rats. The species and sex of experimental animals chosen were based on the potential carcinogenicity "clear evidence" designation from the long-term 2-year toxicology and carcinogenesis study by the National Toxicology Program (2009). Animals also received [¹⁴C]cumene by intravenous injection to investigate the effects of dosing route on cumene disposition and to quantitate biliary excretion of cumene-derived radioactivity. Because the lung has been shown to be a specific target of cumene-mediated toxicity in the mouse and female mice were more susceptible than males (National Toxicology Program, 2009), the metabolism of cumene was investigated in vitro using microsomes prepared from female mouse lung and liver tissues.

Metabolism in female rat lung and liver microsomes 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). Alkamuls-EL 620 (>99% castor oil ethoxylates) was obtained from Rhodia (Cranbury, NJ). Carbo-Sorb E, Permafluor E⁺, and Ultima Gold scintillation cocktail were purchased from PerkinElmer 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-phenylpropionaldehyde, 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/elution ratio. A Luna C18 column (5 μm, 4.6 × 150 mm; Phenomenex, Torrance, CA) was used for disposition studies. The mobile phase included H₂O (solvent A) and acetonitrile (solvent B). The elution began with a linear gradient from 10% B to 95% B over 15 min and 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 × 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 acetonitrile (solvent B). The elution began with a linear gradient from 0% B to 40% B over 30 min, then a linear gradient to 95% B over 10 min, and a linear gradient back to 0% B over 5 min at a flow rate of 1.5 ml/min. The temperature of the column compartment was not maintained, and the UV detection was set at 254 or 210 nm.

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

¹H NMR spectra were acquired on a 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
Target dose of cumene in disposition studies

Dose Route	Male Rat	Mouse	
		Male	Female
		mg/kg	
Intravenous	1.4	10	10
Oral	1.4, 14, 140	10, 50, 100, 1000	10, 150, 1000

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 unlabeled cumene to [^{14}C]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 $\pm$ S.D. for the concentrations and amounts of [^{14}C] administered to rats were 1.7 ± 0.3 mg (92 ± 14 μCi)/kg, 18 ± 1 mg (90 ± 4 μCi)/kg, or 149 ± 10 mg (102 ± 7 μCi)/kg. The target doses administered to mice ($n = 4$) by gavage were 10, 50, 100, or 1000 mg/kg for males and 10, 150, or 1000 mg/kg for females. The means $\pm$ S.D. for the concentrations and amounts of [^{14}C] 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 unlabeled cumene to [^{14}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 $\pm$ S.D. for the concentrations and amounts of [^{14}C] administered to rats were 1.1 ± 0.0 mg (55 ± 2 μCi)/kg and 2.1 ± 0.1 mg (43 ± 2 μCi)/kg to noncannulated and BDC rats. The target dose administered by the intravenous route to three mice per treatment group was 10 mg/kg. The means $\pm$ S.D. for the concentrations and amounts of [^{14}C] administered to mice were 7 ± 0 mg (5 ± 0 μCi)/kg for males and 6 ± 2 mg (4 ± 1 μCi)/kg for females.

Sample Collection and Analysis. For single-dose studies, urine was collected and chilled by dry ice at 6, 12, and 24 h 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}C] excreted in urine. Bile was collected from the BDC rats at 0.25, 1, 2, 3, 4, 5, 6, 12, and 24 h. Triplicate aliquots of urine, bile, and cage rinse were mixed with Ultima Gold scintillation cocktail and counted for [^{14}C] content in a 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 Packard 307 Biological Sample Oxidizer, using Carbo-Sorb E for trapping [$^{14}\text{CO}_2$] and Permafluor E $^+$ as the scintillation cocktail. The samples were then counted in the LSC for determination of [^{14}C] content.

Cumene-derived radioactivity expired as volatile organic compounds (VOCs) and CO_2 was collected by passing the air (flow = 200–600 ml/min) from the metabolism cage through a series of traps. VOCs and CO_2 were not collected in the repeat-dose studies, and CO_2 was collected only in selected single-dose treatment groups. The first two traps contained ethanol or isopropanol (60 ml each) to collect VOCs, and the following two traps contained 1 N NaOH (350 ml each) to collect CO_2 . The first ethanol trap was chilled with wet ice and the second trap with a dry ice-isopropanol slurry to inhibit evaporation of the solvents. The isopropanol and NaOH traps were chilled with wet ice. All traps were changed at 6 and 12 h after dosing. Duplicate aliquots of trapping solution were weighed individually into separate scintillation vials

containing Ultima Gold scintillation cocktail and analyzed for [^{14}C] content in the LSC.

Bile was collected from the BDC rats at 0.25, 1, 2, 3, 4, 5, 6, 12, and 24 h after administration of cumene. All samples were stored at -20°C before analysis.

Blood was collected into a heparinized syringe by cardiac puncture from anesthetized animals 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 as 7.0, 40.4, and 19.0% of the body weight for rats and 7.0, 38.4, and 16.5% for mice. The following entire organs were collected and weighed: brain, lung, heart, spleen, kidneys, urinary bladder, uterus, liver, thyroid, stomach with contents, small intestine with contents, cecum with contents, and the remaining large intestine with contents. Blood, adipose, muscle, skin, brain, lung, heart, spleen, kidney, testes/uterus, urinary bladder, thyroid, and aliquots of homogenized liver were combusted and counted as described above for fecal homogenates. The stomach and small intestines with their contents, the cecum and the remaining large intestine with their contents, and the residual carcass were solubilized in 2 N NaOH in ethanol. For the intravenous study, the tail was removed, solubilized in 2 N NaOH in ethanol, and analyzed for residual [^{14}C]. Once dissolved, these samples were neutralized with nitric acid and bleached with H_2O_2 (30%). Three aliquots of these samples were weighed into scintillation vials containing Ultima Gold scintillation cocktail and analyzed for [^{14}C] in the LSC.

Anesthesia and Euthanasia. At the end of all studies, animals were administered a sodium pentobarbital-based solution by 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 -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 N_2 to remove acetonitrile and then lyophilized in a model 77510 FreezeZone 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, 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}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 MgCl_2 , 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 $\mu\text{Ci}/\mu\text{l}$, 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 , 283.3 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 μ l, 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 N_2 , 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 [ESI(-)-MS: m/z 206 $[M - H]^-$; ESI(-)-MS/MS of m/z 206: m/z 162 $[M - H] - CO_2^-$; ESI(+)-MS: m/z 208 $[M + H]^+$; ESI(+)-MS/MS of m/z 208: m/z 190 $[M + H - H_2O]^+$, 133 $[M + H - glycine]^+$, and 105 $[M + H - glycine - CO]^+$]; and NMR spectra [1H NMR (D_2O): δ 7.46–7.39 (m, 5H, phenyl-H), 3.96 (AB quartet, J = 17.5 Hz, 2H, Gly α -CH $_2$), 3.88 (q, J = 7.0 Hz, 1H, 2-CH), and 1.53 (d, J = 7.0 Hz, 3H, 3-CH $_3$)] confirmed the identity of the 2-phenylpropionylglycine.

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

Results

Excretion of Cumene-Derived Radioactivity. The excretion data from all [^{14}C]cumene studies are shown in Table 2. Approximately 70 to 80% of the total ^{14}C 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 ^{14}C 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 ^{14}C 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 $^{14}CO_2$ in all groups surveyed was negligible (data not shown). A small, but measurable quantity of ^{14}C was excreted as VOCs after single-dose administration of cumene to rats.

Recovery of ^{14}C as VOCs from the 140 mg/kg oral dose was higher relative to the lower oral doses ($p = 0.03$). Intravenously injected rats excreted more ^{14}C as VOCs than did rats gavaged with a similar dose. In BDC rats, $37 \pm 14\%$ of an intravenous dose of 1.4 mg/kg was excreted in bile within 24 h of cumene administration.

Mice (male and female) excreted ^{14}C primarily in the urine after administration of [^{14}C]cumene. However, in contrast to rats, little ^{14}C remained in the intestines of mice 24 h after dosing. There was a trend toward decreased excretion in urine with increasing dose; however, 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 ^{14}C 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 $^{14}CO_2$ was negligible; however, female mice excreted less $^{14}CO_2$ ($0.03 \pm 0.01\%$) at the high dose than did males ($1.6 \pm 0.1\%$) ($p = 0.0001$). Females also excreted more ^{14}C as VOCs at the high dose than did males ($p = 0.02$). Little or no sex-related difference in ^{14}C excretion was observed in the range of middle doses (50–150 mg/kg) administered to mice. The pattern of ^{14}C excretion after intravenous administration was similar to that after administration of a similar oral dose to mice. However, the amount of ^{14}C 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 ^{14}C after daily administration of 150 mg/kg for either 3 or 7 consecutive days to female mice.

Tissue Distribution of Cumene-Derived Radioactivity. Across the range of administered single doses, tissues (excluding stomach and intestines) contained less than 1 and 3% of the total dose in mice and rats, respectively, 24 h after dosing (data not shown). In general, the highest concentrations of residual cumene-derived radioactivity de-

TABLE 2
Recovery of ^{14}C after administration of cumene to male rats and mice of both sexes

Data are expressed as mean $\pm$ S.D. of four animals per treatment group.

Route	Frequency ^a	Dose	Time ^b	% Dose in				Total Recovery ^d
				Urine	Feces	VOC	GI Tract ^c	
<i>mg/kg</i>								
<i>h</i>								
Male rat								
Intravenous	S	1.4	24	90.1 ± 5.9	1.2 ± 0.1	4.6 ± 0.9	17.0 ± 5.7	116.0 ± 3.0
Oral	S	1.4	24	77.4 ± 10.1	1.2 ± 0.5	1.3 ± 0.9	21.9 ± 2.0	105.0 ± 12.0
Oral	S	14	24	70.2 ± 3.4	1.2 ± 0.8	1.4 ± 0.3	22.0 ± 4.6	97.4 ± 6.6
Oral	S	140	24	69.8 ± 8.9	0.9 ± 0.1	8.7 ± 5.0	13.8 ± 1.8	96.9 ± 3.5
Oral	S	14	72	85.6 ± 4.0	5.3 ± 1.8	N.D ^f	0.6 ± 0.1	91.2 ± 2.6
Oral	R	14 × 3	24	73.9 ± 2.7	2.0 ± 0.7	N.D	4.4 ± 1.6	82.2 ± 4.1
Oral	R	14 × 7	24	81.3 ± 5.6	1.5 ± 1.1	N.D	1.4 ± 0.7	85.4 ± 4.6
Male mouse								
Intravenous	S	10	24	47.4 ± 6.5	1.8 ± 1.8	2.2 ± 1.0	0.3 ± 0.1	51.7 ± 9.1
Oral	S	10	24	105.0 ± 5.0	4.5 ± 1.0	3.0 ± 3.5	0.5 ± 0.1	113.0 ± 5.0
Oral	S	50	24	90.2 ± 6.1	5.3 ± 4.0	4.5 ± 5.6	0.2 ± 0.1	100.0 ± 9.0
Oral	S	100	24	79.3 ± 9.9	4.0 ± 2.3	0.6 ± 0.2	0.2 ± 0.0	84.0 ± 10.0
Oral	S	1000	24	80.9 ± 6.1	5.2 ± 4.4	13.9 ± 3.7	0.2 ± 0.1	102.0 ± 5.0
Female mouse								
Intravenous	S	10	24	74.0 ± 8.4	1.7 ± 0.6	3.0 ± 0.4	0.4 ± 0.0	79.0 ± 8.3
Oral	S	10	24	93.7 ± 3.4	3.6 ± 1.9	5.2 ± 5.6	0.3 ± 0.1	103.0 ± 1.0
Oral	S	150	24	86.5 ± 5.0	2.5 ± 1.3	2.9 ± 0.8	0.2 ± 0.1	92.1 ± 5.8
Oral	S	1000	24	79.2 ± 5.7	2.0 ± 0.2	21.9 ± 3.6	1.7 ± 0.6	105.0 ± 5.0
Oral	R	150 × 3	24	80.1 ± 11.1	4.7 ± 1.4	N.D	0.2 ± 0.1	85.0 ± 10.3
Oral	R	150 × 7	24	77.2 ± 7.8	4.7 ± 1.4	N.D	0.1 ± 0.0	87.7 ± 7.7

GI, 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 $^{14}CO_2$ collected in some studies and all surveyed tissues listed under *Materials and Methods*, including the digested carcasses.

TABLE 3

Distribution of ^{14}C in selected tissues after administration of single doses of cumene to male rats and mice of both sexesEach value represents the mean $\pm$ S.D. of three to four animals per treatment group.

Route	Dose	Time	Blood Conc.	Liver		Kidney		Lung	
				Conc.	T/B	Conc.	T/B	Conc.	T/B
	mg/kg	h	nmol-Eq/g	nmol-Eq/g		nmol-Eq/g		nmol-Eq/g	
Male rat									
Intravenous	1.4	24	0.17 $\pm$ 0.03	1.1 $\pm$ 0.3	6.2 $\pm$ 1.5	1.3 $\pm$ 0.3	7.9 $\pm$ 0.9	0.24 $\pm$ 0.02	1.4 $\pm$ 0.3
Oral	1.4	24	0.37 $\pm$ 0.10	2.2 $\pm$ 0.6	5.9 $\pm$ 0.5	2.8 $\pm$ 1.2	7.8 $\pm$ 3.2	0.33 $\pm$ 0.09	0.91 $\pm$ 0.22
Oral	14	24	3.8 $\pm$ 0.3	22 $\pm$ 3	5.9 $\pm$ 1.4	33 $\pm$ 3	8.6 $\pm$ 1.7	6.1 $\pm$ 2.6	1.6 $\pm$ 0.7
Oral	140	24	35 $\pm$ 6	146 $\pm$ 38	4.1 $\pm$ 0.6	279 $\pm$ 30	8.0 $\pm$ 0.7	26 $\pm$ 6	0.74 $\pm$ 0.05
Oral	14	72	0.30 $\pm$ 0.07	1.1 $\pm$ 0.3	3.8 $\pm$ 0.5	1.4 $\pm$ 0.5	4.5 $\pm$ 0.8	0.48 $\pm$ 0.05	1.6 $\pm$ 0.4
Male mouse									
Intravenous	10	24	0.13 $\pm$ 0.03	0.67 $\pm$ 0.11	5.2 $\pm$ 0.5	0.66 $\pm$ 0.03	5.2 $\pm$ 1.1	1.1 $\pm$ 0.1	9.1 $\pm$ 2.5
Oral	10	24	0.40 $\pm$ 0.04	2.6 $\pm$ 0.8	6.5 $\pm$ 1.6	3.2 $\pm$ 1.2	8.1 $\pm$ 3.4	1.4 $\pm$ 0.9	3.6 $\pm$ 2.5
Oral	50	24	0.61 $\pm$ 0.18	4.5 $\pm$ 0.8	7.8 $\pm$ 2.6	3.0 $\pm$ 1.1	5.3 $\pm$ 2.3	2.5 $\pm$ 0.6	4.4 $\pm$ 1.8
Oral	100	24	1.8 $\pm$ 0.2	7.4 $\pm$ 4.6	4.3 $\pm$ 2.8	7.9 $\pm$ 5.2	4.6 $\pm$ 3.0	5.8 $\pm$ 3.2	3.3 $\pm$ 1.9
Oral	1000	24	9.4 $\pm$ 2.1	41 $\pm$ 9	4.4 $\pm$ 0.4	71 $\pm$ 35	7.3 $\pm$ 2.2	21 $\pm$ 9	2.4 $\pm$ 1.3
Female mouse									
Intravenous	10	24	0.24 $\pm$ 0.08	1.3 $\pm$ 0.4	5.3 $\pm$ 0.7	0.58 $\pm$ 0.20	2.4 $\pm$ 0.1	1.7 $\pm$ 0.6	6.9 $\pm$ 0.9
Oral	10	24	0.44 $\pm$ 0.04	2.4 $\pm$ 0.0	5.5 $\pm$ 0.4	0.93 $\pm$ 0.13	2.1 $\pm$ 0.3	0.85 $\pm$ 0.18	2.0 $\pm$ 0.5
Oral	150	24	1.8 $\pm$ 0.5	7.6 $\pm$ 1.7	4.7 $\pm$ 2.1	4.0 $\pm$ 0.9	2.3 $\pm$ 0.4	7.9 $\pm$ 1.2	4.6 $\pm$ 0.8
Oral	1000	24	38 $\pm$ 15	101 $\pm$ 32	2.7 $\pm$ 0.4	139 $\pm$ 109	4.0 $\pm$ 2.6	101 $\pm$ 57	2.6 $\pm$ 0.6

T/B, tissue/blood ratio.

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, 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 ^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

Tissue	Male Rat				Female Mouse			
	1 Dose		7 Doses		1 Dose		7 Doses	
	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B
Adipose	nmol-Eq/g		nmol-Eq/g		nmol-Eq/g		nmol-Eq/g	
Urinary Bladder	12 ± 7	3.1 ± 1.7	14 ± 9	2.6 ± 2.0	1.4 ± 0.5	0.89 ± 0.56	2.9 ± 1.1	0.52 ± 0.23
	72 ± 38	19 ± 12	971 ± 1126	152 ± 154	1.8 ± 0.3	1.1 ± 0.3	11 ± 10	2.1 ± 2.1
Blood	3.8 ± 0.3	Unity	5.8 ± 1.1*	Unity	1.8 ± 0.5	Unity	5.7 ± 0.7*	Unity
Brain	1.4 ± 0.2	0.37 ± 0.04	3.2 ± 1.2	0.56 ± 0.25	1.1 ± 0.4	0.65 ± 0.22	2.3 ± 0.3*	0.41 ± 0.05
Heart	2.4 ± 0.3	0.63 ± 0.06	4.0 ± 1.2	0.69 ± 0.10	1.4 ± 0.1	0.85 ± 0.23	3.7 ± 0.7*	0.65 ± 0.09
Kidney	33 ± 3	8.6 ± 1.7	60 ± 27	10 ± 4	4.0 ± 0.9	2.3 ± 0.4	16 ± 2*	2.8 ± 0.2
Liver	22 ± 3	5.9 ± 1.4	31 ± 9	5.4 ± 1.1	7.6 ± 1.7	4.7 ± 2.1	26 ± 4*	4.6 ± 0.9
Lung	6.1 ± 2.6	1.6 ± 0.6	6.6 ± 1.8	1.1 ± 0.1	7.9 ± 1.2	4.6 ± 0.8	19 ± 1*	3.4 ± 0.4
Muscle	1.0 ± 0.5	0.27 ± 0.13	2.8 ± 0.1*	0.49 ± 0.07	0.87 ± 0.12	0.51 ± 0.13	2.4 ± 0.4*	0.43 ± 0.07
Skin	2.5 ± 0.5	0.66 ± 0.17	5.8 ± 1.8*	1.0 ± 0.3	4.7 ± 2.9	2.5 ± 1.1	7.7 ± 2.0	1.4 ± 0.4
Spleen	2.4 ± 0.8	0.62 ± 0.17	5.4 ± 1.4*	0.94 ± 0.23	1.6 ± 0.3	0.94 ± 0.12	4.1 ± 0.4*	0.72 ± 0.12
Thyroid	0.0 ± 6.5	0.1 ± 1.8	3.8 ± 1.9	0.63 ± 0.22	8.9 ± 7.0	5.3 ± 3.5	N.D	N.A.
Testes	1.3 ± 0.4	0.33 ± 0.12	9.1 ± 7.0	1.6 ± 1.3	1.3 ± 0.0	0.78 ± 0.21	4.4 ± 2.7	0.90 ± 0.54
							15 ± 22	2.5 ± 3.2

TT/B, tissue/blood ratio; N.D., not detected; the average concentration was <0 after background correction; N.A., not applicable.

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

Statistically higher ($p \leq 0.05$) in the same tissues from the seven-dose study versus the three-dose study.

Fig. 2. Cumene-derived radioactivity (nanomole equivalents per gram of tissue) in blood, liver, kidney, and lung of male rats 24 h after one, three, or seven daily oral doses (14 mg/kg) of [14 C]cumene (A) and female mice 24 h after one, three, or seven daily oral doses (150 mg/kg) of [14 C]cumene (B). Data are expressed as the mean $\pm$ S.D. of four animals per treatment group.

SO₃ (80), a dihydroxycumene monosulfate. The NMR spectrum of **M2** showed two CH₃ groups as a singlet at δ 1.55, consistent with hydroxylation at the 2-position of the isopropyl side chain. Because both CH₃ groups were intact, the second hydroxylation must have occurred at the phenyl ring. The coupling of the aromatic protons in **M2** suggested that the ring oxidation was at the *ortho*-position. HPLC reanalysis of the purified metabolite showed not only **M2** at 13.4 min but also another radiolabeled peak at 28.7 min, with a strong UV absorption at 254 nm. This decomposed product was also observed by NMR, which showed resonances at δ 5.41 (s, 1H, olefin H), 5.11 (s, 1H, olefin H), and 2.14 (s, 3H, 3-CH₃); the other signals were not well resolved. The HPLC reanalysis and NMR were consistent with formation of an AMS derivative from dehydration of **M2**. This metabolite was identified as 2-(2-hydroxy-2-propyl)phenylsulfate (**M2**).

M3 with an HPLC retention time of 13.8 min was hydrolyzed by sulfatase, and the hydrolysis product had an HPLC retention time at 17.8 min (Fig. 4D). ESI(–)-MS of **M3** gave a molecular weight of 232, equivalent to cumene (120) + 2 [O] + SO₃ (80), a dihydroxycumene monosulfate. The NMR spectrum of **M3** showed two CH₃ groups as a singlet at δ 1.62 and only four phenyl protons at δ 7.59 and δ 7.34 as two doublets, consistent with hydroxylation at the 2-position of the isopropyl side chain and the *para*-position of the phenyl ring. HPLC reanalysis of the purified metabolite showed not only **M3** at

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.

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] + glucuronide (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-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

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 male mouse dosed orally with a single 1000 mg/kg dose (B), urine (0–24 h) of a female 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).

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-CH_3 and 3-CH_3), in agreement with hydroxylation of cumene at the 2-position of the isopropyl side chain followed

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-phenylpropanthiol anion (*m/z* 167) and 2-acetylaminocrylate (*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 monohydroxycumene glucuronide [cumene (120) + [O] + glucuronide (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 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 $2.0 \pm 0.2\%$ ($n = 3$) 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 ^{14}C as VOCs was dependent on dose, sex, species, and route of administration. Expiration of ^{14}C VOCs increased at the high doses, especially in mice, implying saturation of specific metabolic pathways. Female mice excreted more ^{14}C as VOCs and CO_2 ($p = 0.05$) and retained more ^{14}C in tissues ($p = 0.0001$) than did males at the high dose, indicating 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

Data are percentage of all radiolabeled peaks; mean $\pm$ S.D.; $n = 4$.

Metabolite	Normal Male Rat Urine				BDC Male Rat Bile (1.4 mg/kg i.v.)
	140 mg/kg p.o.	14 mg/kg p.o.	1.4 mg/kg p.o.	1.4 mg/kg i.v.	
M1	N.D.	N.D.	N.D.	N.D.	N.D.
M2	Trace ^a	Trace	Trace	Trace	N.D.
M3	11.4 $\pm$ 1.6	8.2 $\pm$ 0.9	7.0 $\pm$ 0.9	4.9 $\pm$ 1.1	N.D.
M4	5.6 $\pm$ 0.5	5.2 $\pm$ 0.7	5.6 $\pm$ 1.1	5.5 $\pm$ 0.7	N.D.
M5	2.6 $\pm$ 0.3	2.5 $\pm$ 0.4	2.2 $\pm$ 1.5 ^b	3.0 $\pm$ 0.3	N.D.
M6	1.6 $\pm$ 0.0	N.D.	N.D.	N.D.	4.5 $\pm$ 0.6 ^c
M7	17.8 $\pm$ 1.0	20.1 $\pm$ 0.6	19.3 $\pm$ 0.8	17.4 $\pm$ 0.4	11.8 $\pm$ 2.8 ^c
M8	16.4 $\pm$ 2.0	12.1 $\pm$ 0.8	12.1 $\pm$ 0.3	10.8 $\pm$ 1.6	N.D.
M9 + M10^d	38.1 $\pm$ 2.2	47.0 $\pm$ 1.3	48.4 $\pm$ 3.6	49.6 $\pm$ 3.0	73.2 $\pm$ 2.6 ^c
M11	N.D.	N.D.	N.D.	N.D.	N.D.
M12 + M13	4.8 $\pm$ 0.5	4.9 $\pm$ 0.5	4.0 $\pm$ 1.0	6.3 $\pm$ 1.7	5.4 $\pm$ 0.4 ^c
M14	1.6 ^e	Trace	1.8 ^c	Trace	N.D.
M15	N.D.	N.D.	N.D.	N.D.	N.D.
M16	2.1 $\pm$ 0.5	Trace	Trace	2.3 $\pm$ 0.2 ^b	2.9 ^e

N.D., not detected.

^a A trace amount was observed but not quantified.

^b $n = 2$.

^c $n = 3$.

^d **M10** is a minor metabolite coeluted with **M9**.

^e $n = 1$.

TABLE 6

Percentage of dose of cumene metabolites in cumulative 0–24 h mouse urine

Data are percentage of all radiolabeled peaks; mean $\pm$ S.D.; $n = 4$.

Metabolite	Male Mouse Urine			Female Mouse Urine		
	1000 mg/kg p.o.	100 mg/kg p.o.	10 mg/kg p.o.	1000 mg/kg p.o.	150 mg/kg p.o.	10 mg/kg p.o.
M1	Trace ^a	N.D	N.D	3.0 $\pm$ 0.3	2.9 $\pm$ 1.0	1.8 $\pm$ 0.3
M2	Trace	N.D	N.D	4.4 $\pm$ 1.0	3.4 $\pm$ 0.2	N.D
M3	N.D	N.D	N.D	Trace	Trace	N.D
M4	N.D	N.D	N.D	Trace	N.D	N.D
M5	3.0 $\pm$ 0.1	6.3 $\pm$ 0.6	8.4 $\pm$ 0.3	5.8 $\pm$ 1.3	16.7 $\pm$ 1.9	19.1 $\pm$ 1.4
M6	4.4 $\pm$ 0.4	2.9 $\pm$ 0.4	3.1 $\pm$ 0.8	4.2 $\pm$ 0.5	2.6 $\pm$ 0.7	2.5 $\pm$ 0.5
M7	16.9 $\pm$ 0.5	11.9 $\pm$ 0.4	8.6 $\pm$ 0.9	16.5 $\pm$ 1.3	9.0 $\pm$ 1.8	6.1 $\pm$ 0.5
M8	12.8 $\pm$ 2.2	14.4 $\pm$ 0.8	15.7 $\pm$ 0.9	20.4 $\pm$ 0.8	12.9 $\pm$ 0.7	11.4 $\pm$ 0.9
M9	42.8 $\pm$ 2.4	39.3 $\pm$ 3.4	33.5 $\pm$ 2.2	36.8 $\pm$ 2.1	35.1 $\pm$ 2.1	29.8 $\pm$ 0.9
M11	11.0 $\pm$ 0.5	6.4 $\pm$ 1.3	5.1 $\pm$ 0.9	2.8 $\pm$ 0.2	2.9 $\pm$ 0.4	3.7 $\pm$ 0.4
M12^b + M13	5.8 $\pm$ 1.5	2.1 $\pm$ 0.2	1.6 $\pm$ 0.1	2.1 ^c	2.3 $\pm$ 0.4	1.5 $\pm$ 0.2
M14	N.D	1.5 ^c	N.D	N.D	N.D	N.D
M15	N.D	1.6 ^c	N.D	N.D	N.D	N.D
M16	Trace	N.D	N.D	Trace	N.D	N.D

N.D., not detected.

^a A trace amount was observed but not quantified.^b Only a trace amount of **M12** was observed in mouse urine.^c $n = 1$.

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 $\alpha_{2\text{u}}$ -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*-(+)-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

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).

TABLE 7

*Percentage of cumene and metabolites in microsomal incubations*Data are percentage of all radiolabeled peaks; mean $\pm$ S.D.; $n = 4$.

Metabolite	Female Mouse		Female Rat	
	Lung Microsomes	Liver Microsomes	Lung Microsomes	Liver Microsomes
2-Phenyl-2-propanol (M14)	37.7 $\pm$ 2.3	15.5 $\pm$ 3.3	10.5 $\pm$ 2.0	7.7 $\pm$ 0.8
2-Phenyl-1-propanol (M15)	3.4 $\pm$ 0.8	N.D	4.4 $\pm$ 1.4	N.D
AMS	6.2 $\pm$ 0.5	3.4 $\pm$ 0.4	0.7 $\pm$ 0.9 ^a	1.3 $\pm$ 0.4
Cumene	52.8 $\pm$ 2.4	81.2 $\pm$ 3.4	84.3 $\pm$ 3.1	91.0 $\pm$ 0.7

N.D., not detected.

^a AMS was detected in two (1.8 and 1.1%) of four incubations.

ruled out. 2-Phenylpropionic acid (**M16**) was further metabolized to a glucuronide conjugate (**M10**), predominantly in rats, and a glycine conjugate (**M11**), predominantly in mice. **M11** was more abundant in male 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* 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. 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/bronchiolar cytotoxicity in humans would be much less than in mice or even rats that have not shown evidence of lung cytotoxicity.

AMS exposure also has resulted in increased accumulation of hyaline droplets in the renal tubules of male rats (Morgan et al., 1999). Hyaline droplets, which contain α_{2u} -globulins, can lead to granular casts and single cell necrosis, increased cell division and tubule hyperplasia, and finally renal tubule adenoma and carcinoma (Rodgers 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 α_{2u} -globulin is a male rat-specific protein that is not present in female rats, male or female mice, or humans (Flamm and Lehman-McKeeman, 1991; Lehman-McKeeman 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 α_{2u} -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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Authorship Contributions

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

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

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