Metabolism and Disposition of \( n \)-Butyl Glycidyl Ether in F344 Rats and B6C3F1 Mice


Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

Received May 29, 2007; accepted September 12, 2007

ABSTRACT:
The disposition of \( [14\text{C}] \)-labeled \( n \)-butyl glycidyl ether (BGE, 3-butoxy-1,2-epoxypropane) was studied in rats and mice. The majority of a single p.o. dose (2–200 mg/kg) was excreted in urine (rats, 84–92%; mice, 64–73%) within 24 h. The rest of the dose was excreted in feces (rats, 2.6–7.7%; mice, 5.3–12%) and in expired air of a single p.o. dose (2–200 mg/kg) was excreted in urine (rats, toxy-1,2-epoxypropane) was studied in rats and mice. The majority undergone further \( \alpha \)-1 oxidation to form a 3'-hydroxybutytox substitution. One urinary metabolite was from \( \alpha \) oxidation of 3-butoxy-1-(N-acetylcystein-S-yl)-2-propanol to yield the corresponding carboxylic acid. Oxidative deamination of 3-butoxy-1-(cystein-S-yl)-2-propanol gave the corresponding \( \alpha \)-keto acid and \( \alpha \)-hydroxy acid metabolites that were present in mouse urine but not in rat urine. An in vitro incubation of BGE with GSH showed that the conjugation occurred only at the C-1 position with or without the addition of GSH S-transferase.

Epoxy resins are used in electronics, coatings, and a variety of other applications. They are generally prepared from a phenol, commonly bisphenol A, and a reactive epoxide such as epichlorohydrin. The properties of the resin are modified by including other phenols or epoxides. \( n \)-Butyl glycidyl ether (BGE, 3-butoxy-1,2-epoxypropane) is one of the modifiers used to reduce the viscosity of the resin (Kirk-Othmer, 2005). BGE is a high production volume chemical with production of more than 1 million pounds produced yearly in the United States [National Toxicology Program (NTP), 2004]. It has been selected for study by the NTP as an example of the aliphatic glycidyl ethers class.

The acute toxicity of BGE is low. The oral LD\(_{50}\) is reported to be 1.53 and 2.26 g/kg in mice and rats, respectively. Inhalation exposure to as much as 200 ppm for 8 h appeared to affect the central nervous system, cause congestion of the central zones of the liver, and result in pneumonitis and irritation of the lungs. BGE is positive in the Ames test without the addition of S9 (NTP, 2004). There are no chronic toxicity studies reported for BGE, however, 2-year studies on an olefinic and an aromatic glycidyl ether have been conducted. Chronic exposure to 5 or 10 ppm allyl glycidyl ether by inhalation resulted in some (male mice), equivocal (male rat, female mice), or no (female rats) evidence of carcinogenic activity (NTP, 1990). Chronic exposure to diglycidyl resorcinoil ether by gavage administration resulted in increases in benign and malignant lesions of the forestomach in male and female mice (50 or 100 mg/kg) and rats (25 or 50 mg/kg) (NTP, 1986).

The absorption, tissue distribution, metabolism, and excretion of BGE in rats have been investigated, but the data are limited. Male rats excreted 87% of a p.o. dose (\( [14\text{C}] \)-BGE, 20 mg/kg) in urine within 24 h (Eadsforth et al., 1985). Thin layer chromatography analysis of the urine revealed more than 10 metabolites, although only three major metabolites, including 3-butoxy-2-hydroxypropionic acid (9%), \( O \)-butyl-N-acetylsamine, butoxyacetic acid, 2-butoxyethanol, and 3-butoxy-1-(N-acetylcystein-S-yl)-2-propanol, the mercapturic acid metabolite derived from conjugation of glutathione (GSH) with BGE at the C-1 position. Some of these metabolites underwent further \( \alpha \)-1 oxidation to form a 3'-hydroxybutytox substitution. One urinary metabolite was from \( \alpha \) oxidation of 3-butoxy-1-(N-acetylcystein-S-yl)-2-propanol to yield the corresponding carboxylic acid. Oxidative deamination of 3-butoxy-1-(cystein-S-yl)-2-propanol gave the corresponding \( \alpha \)-keto acid and \( \alpha \)-hydroxy acid metabolites that were present in mouse urine but not in rat urine. An in vitro incubation of BGE with GSH showed that the conjugation occurred only at the C-1 position with or without the addition of GSH S-transferase.

This research was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences.

This article was published in Drug Metabolism and Disposition on June 28, 2017.

Materials and Methods

Chemicals. \([n\text{-Butyl-1-}^{14}\text{C}]-\text{BGE}\) (specific activity, 55 mCi/mmol; radiolabeling purity, 99.7%) was obtained from Moravek Biochemicals (Brea, CA). Unlabeled BGE (parity, 95%), 2-butoxyethanol, glutathione (GSH), GSH S-transferase (GST) (75% pure) from rat liver, and \( \beta \)-glucuronidase from human urine were used.

Received May 29, 2007; accepted September 12, 2007

ABBREVIATIONS: BGE, \( n \)-butyl glycidyl ether; NTP, National Toxicology Program; GSH, glutathione; GST, glutathione S-transferase; HPLC, high-performance liquid chromatography; ESI, electrospray ionization; MS, mass spectrometry; MS/MS, tandem mass spectrometry; LC/MS, liquid chromatography/mass spectrometry.
bovine liver (10 million units/g solid) were purchased from Sigma-Aldrich (St. Louis, MO).

Instruments. 1H NMR spectra were acquired on a Varian, Inc. Gemini 300 MHz NMR spectrometer (Palo Alto, CA). Chemical shifts are reported in parts per million relative to D2O at 4.80 ppm. High-performance liquid chromatography (HPLC) analysis of urine (10–20 μl) was carried out on a Beckman System Gold 126 Solvent Module pump (Beckman Instruments, Fullerton, CA), a 168-photodiode array detector, and an IN/US (Tampa, FL) β-RAM flow detector equipped with a liquid cell (500 μl) for radiochemical detection. Liquid scintillation fluid (In-Flow ES, INUS) was delivered in a 3:1 scintillator/centrart volume. A Varian, Inc. Inertis C18 5-μm column (4.6 × 250 mm) was used for all the studies. The solvent system consisted of solvent A, 0.1% trifluoroacetic acid in H2O, and solvent B, CH3CN, and a gradient from 100% A and 0% B to 65% A and 35% B over 28 min, then held at 35% B for 5 min at a flow rate of 1.5 ml/min. This HPLC system was used for detection of radiolabeled peaks and for isolation of the metabolites, and will be referred to as HPLC under Results and Discussion. Electrospray ionization mass spectra (ESI/MS) were obtained on a Thermo Finnigan LCQ Advantage Max ion trap mass spectrometer (Waltham, MA). Tandem mass spectra (ESI/MS/MS) were produced by collision-induced dissociation of selected parent ions with helium in the mass analyzer. Samples were dissolved in CH3OH/H2O (1:1) and introduced into the mass spectrometer through direct infusion (12.5 μl/min) for either negative ionization [ESI(−)/MS or ESI(−)/MS/MS] or positive ionization [ESI(+)/MS or ESI(+)/MS/MS] analysis. The heated capillary was maintained at 250°C and the source voltage at 5 kV. Liquid chromatography/mass spectrometry (LC/MS) of the collected urine (1–4 μl) was carried out on an Agilent Technologies (Santa Clara, CA) 1100 HPLC equipped with an Agilent G1315B DAD photodiode array detector and connected with the LCQ ion trap mass spectrometer. A Varian, Inc. Polaris C18 3-μm column (20 × 100 mm) was used for all the LC/MS analyses. The solvent system consisted of solvent A, 0.1% formic acid in H2O, and solvent B, 0.1% formic acid in CH3CN, and a gradient from 95% A and 5% B to 65% A and 35% B over 30 min at a flow rate of 200 μl/min. The MS was run in the negative ionization mode [ESI(−)/MS or ESI(−)/MS/MS], the heated capillary maintained at 300°C, and the source voltage at 5 kV. This HPLC system was used for identification of the BGE metabolites by MS analysis and will be referred to as LC/MS under Results and Discussion.

Animals and Treatments. Male (11.5–12.5 weeks old, 233–276 g) and female (13 weeks old, 179–196 g) F344 rats were obtained from Charles River Laboratories (Kingston, NY). Male (8 weeks old, 24–27 g) and female (7–9 weeks old, 19–22 g) B6C3F1 mice were obtained from Charles River Laboratories (Raleigh, NC). Male rats and female mice (n = 4 or 5/treatment groups) were dosed with [14C]-labeled BGE (2, 20, or 200 mg/kg; 37–56 μCi/kg) by gavage. Female rats and male mice (n = 4 or 5/treatment groups) were dosed with [14C]-labeled BGE (200 mg/kg; 53–55 μCi/kg) by gavage. All the p.o. dose solutions were in corn oil, 5 ml/kg for rats, and 10 ml/kg for mice. Animals were housed individually in metabolism cages allowing for the collection of urine, feces, and expired air and were provided with food (National Institutes of Health #13) and distilled water ad libium. Animals were euthanized by CO2 asphyxiation 24 h postdosing from male rats dosed p.o. with BGE (200 mg/kg) was analyzed by the HPLC with radiochemical detection to reveal 13 radiolabeled peaks (Fig. 1A, 1–13). The metabolites were not observed by UV detection, so the isolation of these urinary metabolites was accomplished by collection of HPLC fractions at 1-min intervals between 8 and 28 min. The solvents were evaporated by a Speed-Vac (Thermo Savant, Waltham, MA), and the metabolites were characterized by MS and 1H NMR. The spectral data of metabolites 1 through 13 were obtained, and metabolites 1 through 12 were identified as the structures shown in Fig. 2.

Metabolite 1 had an HPLC retention time of 8.7 min. 1H NMR (D2O): δ 3.98 to 3.83 (m, 2 H, 1-CH2), 3.09 (dd, J = 6.6 Hz, 2H 2O), 1.62 (q, 7.5 Hz, 2 H, CH3); the other protons were not well resolved or overlapped with the signals of endogenous compounds.

Metabolite 2 had HPLC retention times of 9.6 and 10.1 min. ESI(−)/MS/MS: m/z 177 [M – H]−, 133 [M – H – CO2]−, 89 [3′-hydroxybutoxide]−, and 87 [2-hydroxyacrylate]−. 1H NMR (D2O) of the major isomer: δ 1.73 (q, 6.6 Hz, 2H, butoxy 4-CH2), 1.18 (d, J = 6.5 Hz, 3H, butoxy 4′-CH3); the minor isomer: δ 1.62 (q, 7.5 Hz, 2H, butoxy 2′-CH3), 1.11 (d, J = 7.2 Hz, 3H, butoxy 4′-CH3); the other protons were not well resolved or overlapped with the signals of endogenous compounds.

Metabolite 2 had HPLC retention times of 9.6 and 10.1 min. ESI(−)/MS/MS: m/z 177 [M – H]−, 133 [M – H – CO2]−, 89 [3′-hydroxybutoxide]−, and 87 [2-hydroxyacrylate]−. 1H NMR (D2O) of the major isomer: δ 1.73 (q, 6.6 Hz, 2H, butoxy 4-CH2), 1.18 (d, J = 6.5 Hz, 3H, butoxy 4′-CH3); the minor isomer: δ 1.62 (q, 7.5 Hz, 2H, butoxy 2′-CH3), 1.11 (d, J = 7.2 Hz, 3H, butoxy 4′-CH3); the other protons were not well resolved or overlapped with the signals of endogenous compounds.
Fig. 2. Proposed metabolic pathways of BGE in rats and mice.

β-CH₃), 2.92 (dd, J = 13.6, 7.6 Hz, 1H, cyc β-CH₃), 2.79 (dd, J = 13.8, 5.3 Hz, 1H, 1′-CH₂), 2.64 (dd, J = 13.8, 6.7 Hz, 1H, 1′-CH₂), 2.06 (s, 3H, COCH₃), 1.74 (q, J = 6.9 Hz, 2H, butoxyl 2′-CH₂), and 1.19 (d, J = 6.6 Hz, 3H, butoxyl 4′-CH₃).

Metabolite 5 had an HPLC retention time of 14.3 min. ES(−)/MS/MS: m/z 322 [M − H]−, 219 [M − 4-hydroxybutanoic acid]−, 193 [M − H − 2-acetylaminoacrylic acid]−, 175 [M − H − 2-acetylaninoacrylic acid − H₂O]−, 128 [2-acetylaninoacrylate]−, and 103 [4-hydroxybutanoate]−. 1H NMR (D₂O): δ 3.93 (br, s, 1H, 2-C₃H₂), 3.63 to 3.50 (m, 4H, 3-CH₂, and butoxy 1′-CH₂), 3.09 (dd, J = 12.6, 3.7 Hz, 1H, cyc β-CH₃), 2.92 (dd, J = 13.3, 8.0 Hz, 1H, cyc β-CH₃), 2.79 (dd, J = 14.6, 4.3 Hz, 1H, 1′-CH₂), 2.63 (dd, J = 14.5, 8.2 Hz, 1H, 1′-CH₂), 2.44 (t, J = 7.1 Hz, 2H, butoxyl 3′-CH₂), 2.06 (s, 3H, COCH₃), and 1.95 to 1.82 (m, 2H, butoxyl 2′-CH₂).

Metabolite 6 had an HPLC retention time of 16.2 min. ES(−)/MS/MS: m/z 227 [M − H]−, 153 [M − H − butanol]−, 97 [HSO₄]−, and 81 [H₂SO₄]−. 1H NMR (D₂O): δ 4.49 (quintet, J = 4.8 Hz, 1H, 2-C₃H₂), 3.87 to 3.55 (m, 6H, 1′-CH₃, 3-CH₂, and butoxy 1′-CH₂), 1.56 (quintet, J = 7.4 Hz, 2H, butoxyl 2′-CH₂), 1.34 (sextet, J = 7.4 Hz, 2H, butoxyl 3′-CH₂), and 0.90 (t, J = 7.4 Hz, 3H, butoxyl 4′-CH₃).

Metabolite 7 had an HPLC retention time of 17.3 min. ES(−)/MS/MS: m/z 323 [M − H]−, 305 [M − H − H₂O]−, 287 [M − H − 2 H₂O]−, 175 [glucoronic acid − H₂O]−, 157 [glucoronic acid − 2 H₂O]−, 147 [M − H − 166 (Gluc)]−, or [3-butoxy-2-hydroxy-1-propanol − H]−. 1H NMR (D₂O): δ 4.51 (d, J = 7.7 Hz, 1H, Gluc 1′-CH), 4.06 to 3.96 (m, 1H, 2-C₃H₂), 3.92 to 3.86 (m, 1H, Gluc 5′-CH₃), 3.77 to 3.50 (m, 8H, 1′-CH₃, 3-CH₂, and butoxy 1′-CH₂, and Gluc 2′-CH₂, 4′-CH₂), 3.36 (t, J = 8.2 Hz, 1H, Gluc 3′-CH₃), 1.56 (quintet, J = 7.2 Hz, 2H, butoxyl 2′-CH₂), 1.34 (sextet, J = 7.5 Hz, 2H, butoxyl 3′-CH₂), and 0.89 (t, J = 7.4 Hz, 3H, butoxyl 4′-CH₃).

Metabolite 8 had an HPLC retention time of 19.4 min. ES(−)/MS/MS: m/z 161 [M − H]−, 115 [M − H − COOH]−, and 73 [butoxyl]−. 1H NMR (D₂O): δ 4.30 (dd, J = 4.9, 3.1 Hz, 1H, 2-C₃H₂), 3.83 to 3.67 (m, 2H, 3-CH₂), 3.64 to 3.49 (m, 2H, butoxyl 1′-CH₂), 1.55 (quintet, J = 7.3 Hz, 2H, butoxyl 2′-CH₂), 1.33 (sextet, J = 7.4 Hz, 2H, butoxyl 3′-CH₂), and 0.89 (t, J = 7.3 Hz, 3H, butoxyl 4′-CH₃).

Metabolite 9 had an HPLC retention time of 21.7 min. ES(−)/MS/MS: m/z 202 [M − H]−, 160 [M − H − COCH₃]−, 128 [2-acetylaninoacrylate]−, and 84 [2-acetylaninoacrylate − CO₂]−. 1H NMR (D₂O): δ 4.58 (t, J = 5.0 Hz, 1H, 1′-CH₂), 3.88 to 3.78 (m, 2H, 3′-CH₃), 3.62 to 3.48 (m, 2H, butoxyl 1′-CH₂), 2.06 (s, 3H, COCH₃), 1.59 (quintet, J = 7.2 Hz, 2H, butoxyl 2′-CH₂), 1.32 (sextet, J = 7.4 Hz, 2H, butoxyl 3′-CH₂), and 0.88 (t, J = 7.3 Hz, 3H, butoxyl 4′-CH₃).

Metabolite 10 had an HPLC retention time of 22.4 min. ES(−)/MS/MS: m/z 131 [M − H]−. This ion did not fragment on MS/MS analysis. 1H NMR (D₂O): δ 8.39 (s, 2H, CH₂CO), 3.52 (t, J = 7.3 Hz, 2H, butoxyl 1′-CH₂), 1.57 (quintet, J = 8.2 Hz, 2H, butoxyl 2′-CH₂), 1.34 (sextet, J = 7.6 Hz, 2H, butoxyl 3′-CH₂), and 0.89 (t, J = 7.7 Hz, 3H, butoxyl 4′-CH₃).

Metabolite 11 had an HPLC retention time of 23.9 min. ES(−)/MS/MS: m/z 292 [M − H]−, 250 [M − H − COCH₃]−, 163 [3-butoxy-2-hydroxy-1-propanethiol − H]−, and 128 [2-acetylaninoacrylate]−. 1H NMR (D₂O): δ 4.59 (dd, J = 7.7, 4.7 Hz, 1H, Cys α-CH), 3.95 (quintet, J = 5.7 Hz, 1H, 2-C₃H₂), 3.60 to 3.47 (m, 4H, 3-CH₂, and butoxyl 1′-CH₂), 3.13 (dd, J = 14.1, 4.5 Hz, 1H, cyc β-CH₃), 2.96 (dd, J = 13.8, 8.0 Hz, 1H, cyc β-CH₃), 2.81 (dd, J = 14.1, 5.4 Hz, 1H, 1′-CH₂), 2.65 (dd, J = 13.8, 7.2 Hz, 1H, 1′-CH₂), 2.06 (s, 3H, COCH₃), 1.57 (quintet, J = 7.3 Hz, 2H, butoxyl 2′-CH₂), 1.34 (sextet, J = 7.5 Hz, 2H, butoxyl 3′-CH₂), and 0.89 (t, J = 7.4 Hz, 3H, butoxyl 4′-CH₃).

Metabolite 12 had an HPLC retention time of 26.1 min. 1H NMR (D₂O): δ 3.73 (t, J = 4.6 Hz, 2H, 1′-CH₂), 3.57 (t, J = 5.6 Hz, 2H, 2′-CH₂), 3.52 (t, J = 8.8 Hz, 2H, butoxyl 1′-CH₂), 1.55 (quintet, J = 7.1 Hz, 2H, butoxyl 2′-CH₂),
1.33 (sex: tet, J = 7.3 Hz, 2H, butoxy 3'-CH₂), and 0.89 (t, J = 7.4 Hz, 3H, butoxy 4'-CH₃).

Metabolite 13 had an HPLC retention time of 27.0 min. 1H NMR (D₂O): δ 4.21 (quintet, J = 4.5 Hz, 1H, 2-CH₃), 3.86 to 3.70 (m, 3H, 3-CH₂, and 1-CH₃), 3.61 to 3.50 (m, 3H, butoxy 1'-CH₂, and 1'-CH₃), 1.57 (quintet, J = 7.4 Hz, 2H, butoxy 2'-CH₃), 1.35 (sex: tet, J = 7.7 Hz, 2H, butoxy 3'-CH₂), and 0.90 (t, J = 7.3 Hz, 3H, butoxy 4'-CH₃).

HPLC analysis of urine of female mice administered a 200-mg/kg p.o. dose revealed metabolites with similar retention times as those present in rat urine (metabolites 1–13) and three additional radiolabeled peaks (metabolites 14–16) (Fig. 1B). The HPLC retention times of metabolites 14 to 16 were as follows: 14 (17.7 min), 15 (23.3 min), and 16 (29.0 min). Metabolite 15 had an LC/MS retention time at 20.8 min and an ESI(+)MS/MS spectrum as follows: m/z 251 [M + H]⁺, 233 [M – H – H₂O]⁺, 163 [3-butoxy-2-hydroxy-1-propanethiol – H]⁻, and 87 [2-hydroxyacrylate]⁻. Metabolite 16 had an LC/MS retention time at 25.7 min and an ESI(+)MS/MS spectrum as follows: m/z 249 [M – H]⁻, 205 [M – H – CO₂]⁻, and 163 [3-butoxy-2-hydroxy-1-propanethiol – H]⁻.

Incubation of BGE with GST and GSH. Incubations of [14C]-BGE (1 mM) with GSH (5 mM) were conducted in a 0.1 M potassium phosphate buffer (pH 7.4) using a Tukey-Kramer test. Values were considered statistically significant at p ≤ 0.05.

### Results

**Excretion.** The disposition of BGE in rats and mice 24 h after a single p.o. dose is shown in Table 1. Most of the dose (2–200 mg/kg) administered to male and female rats was excreted in urine (84–92%) within 24 h. The rest of the dose was excreted in feces (2.6–7.7%), expired air (0.1% volatiles and 1.5% CO₂, 20 mg/kg), or remained in the tissues (1.8–4.4%). There is no obvious dose effect on disposition among the range of doses (2–200 mg/kg) in male rats. Female rats have less of the dose remaining in tissues compared with males. Most of the dose given to male and female mice was also excreted in urine (64–73%). Mice appeared to excrete a larger percentage of the dose in feces (5.3–12%), but separation of urine and feces in mouse metabolism cages was not complete, and contamination of urine with feces occurred in some experiments. This resulted in an underestimation of the recovery from urine and overestimation of recovery from feces. As a result of the contamination, some of the urine samples were not useful for metabolic profile analysis. Mice excreted a larger percentage of the p.o. dose in expired air as [14CO₂ (10–18%) and had less remaining in the tissues (1.5–1.7%) than rats within 24 h. Mice that received a 200-mg/kg dose excreted more [14CO₂ than the lower dose treatment (2 and 20 mg/kg). The total recovery was 93 to 98% for rats and 88 to 97% for mice.

**Tissue Distribution.** The BGE-derived radioactivity remaining in tissues 24 h after dosing with 2 to 200 mg/kg accounted for less than 5% of the dose (Table 1). Table 2 summarizes the tissue distribution data 24 h after a single p.o. dose (200 mg/kg) to male and female rats and mice. The concentration of BGE-derived radioactivity in glandular and forestomach is high in mice. Tissue distribution data were obtained for 2 and 20 mg/kg doses for male rats and female mice (data not shown). The distribution was generally dose-proportional, and with the exception of forestomach, the data for the 200-mg/kg dose adequately describe the distribution of lower doses. Forestomach concentration after a p.o. dose of 2, 20, and 200 mg/kg was 42, 530, and 1770 nmol/g in female mice and 9.6, 90, and 323 nmol/g in male rats. Tissues listed under Materials and Methods, but not appearing in Table 2, contained 0.01% or less of the dose for both species and sexes.

**Identification of Urinary Metabolites in Rats.** The urinary metabolite profile from male rats dosed p.o. with BGE (200 mg/kg) is...
shown in Fig. 1A, which adequately describes the urinary metabolite profiles from male rats dosed with 2 and 20 mg/kg and female rats dosed with 200 mg/kg. The spectral data for these 13 metabolites (designated 1–13 in Fig. 1A) were obtained and reported under Materials and Methods, and metabolites 1 through 12 were identified as the structures shown in Fig. 2. Some of these metabolites exist as stereoisomers. C-2 of BGE is an asymmetric center, so BGE exists as two enantiomers. There are up to three chiral centers present at BGE metabolites, so in theory metabolites 8 and 9 could include two enantiomers, metabolites 1 through 3 could include two sets of enantiomers that are diastereomeric, metabolites 5 and 11 could include two diastereomers, and metabolite 4 could include four diastereomers. However, NMR analysis of these metabolites either could not distinguish the diastereomers or show only the major isomer. The HPLC chromatograms in Fig. 1 show more than one isomer for some of these metabolites, but the exact numbers of isomers cannot be established because these stereoisomers are not always adequately separated.

Metabolite 1 did not ionize on ESI/MS analysis, so we were not able to obtain the molecular mass. The NMR data clearly indicate ω-1 oxidation of the butoxy group and eight protons on carbons attached to O. The difference in retention times between the other ω-1 oxidation products and the primary metabolites is 9.6 min (metabolites 2 and 8), 10.7 min (metabolites 3 and 9), and 10.0 min (metabolites 4 and 11). We suspect that metabolite 1 is the ω-1 oxidation product of 3-butoxy-2-hydroxy-1-propanol (metabolite 14), the hydration product of BGE, because metabolite 14 had an HPLC retention time at 17.7 min, a 9.0-min difference in retention times from 1; 2) metabolite 14 contains seven protons on carbons attached to O, and ω-1 oxidation would give a product with eight protons on carbons attached to O; and 3) both metabolites 14 and 1 are phase I metabolites that do not ionize readily. Metabolite 1 is tentatively identified as 3-(3'-hydroxybutoxy)-2-hydroxy-1-propanol.

The two isomeric metabolites 2 have a molecular mass of 178, 16 Da higher than that of 3-butoxy-2-hydroxypropionic acid (metabolite 8), and the NMR result indicates ω-1 oxidation of the butoxy group. The MS fragmentation is consistent with ω-1 oxidation product of 3-hydroxybutanoic acid and 2-acetylamino propionic acid being parts of the molecule. The NMR spectrum contained peaks for seven protons on carbons attached to O, and the NMR result indicates ω-1 oxidation of the butoxy group. The MS fragmentation is consistent with ω-1 oxidation of metabolite 2 and identified as 3-(3'-hydroxybutoxy)-2-hydroxy-1-propanol. The molecular mass (162) of metabolite 8 is consistent with that of 3-butoxy-2-hydroxypropionic acid (metabolite 8), and the NMR result indicates ω-1 oxidation of the butoxy group.

Metabolite 4 has a molecular mass of 309, 16 Da higher than that of 3-butoxy-2-hydroxy-1-(N-acetylcystein-S-yl)-2-propanol (metabolite 11), and the NMR result indicates ω-1 oxidation of the butoxy group. The MS fragmentation is consistent with 3-(3'-hydroxybutoxy)-2-hydroxy-1-propanethiol and 2-acetylamino propionic acid being parts of the molecule. The NMR spectrum contained peaks for seven protons on carbons attached to O, which is in agreement with a diol structure. The chemical shift of the 2-CH is downfield compared with the corresponding proton in metabolite 1, which implies that the sulfure conjugation is likely on 2-OH, but the assignment is not certain. Metabolite 6 is identified as a monosulfate conjugate of 3-butoxy-2-hydroxy-1-propanol.

Metabolite 7 was hydrolyzed by β-glucuronidase to its aglycone (retention time = 17.7 min), which had a similar retention time as that of 3-butoxy-2-hydroxy-1-propanol (14). MS/MS confirms that metabolite 7 is a glucuronide conjugate, and its molecular mass of 228 is equivalent to BGE + H2O + SO3. The NMR spectrum contained peaks for seven protons on carbons attached to O, which is in agreement with a diol structure. The chemical shift of the 2-CH is downfield compared with the corresponding proton in metabolite 1, which implies that the sulfure conjugation is likely on 2-OH, but the assignment is not certain. Metabolite 6 is identified as a monosulfate conjugate of 3-butoxy-2-hydroxy-1-propanol.

Metabolite 8 is a sulfate conjugate, and its molecular mass of 228 is equivalent to BGE + H2O + SO3. The NMR spectrum contained peaks for seven protons on carbons attached to O, which is in agreement with a diol structure. The chemical shift of the 2-CH is downfield compared with the corresponding proton in metabolite 1, which implies that the sulfure conjugation is likely on 2-OH, but the assignment is not certain. Metabolite 6 is identified as a monosulfate conjugate of 3-butoxy-2-hydroxy-1-propanol.

Metabolite 9 is a glucuronide conjugate, and its molecular mass of 324 is equivalent to BGE + H2O + glucuronide. NMR showed several protons on carbons attached to O, which is in agreement with a diol structure and conjugation with a glucuronide. Metabolite 7 is identified as a monoglucuronide conjugate of 3-butoxy-2-hydroxy-1-propanol with the conjugation on either 1-OH or 2-OH.

Metabolite 10 is a glucuronide conjugate, and its molecular mass of 228 is equivalent to BGE + H2O + SO3. The NMR spectrum contained peaks for seven protons on carbons attached to O, which is in agreement with a diol structure. The chemical shift of the 2-CH is downfield compared with the corresponding proton in metabolite 1, which implies that the sulfure conjugation is likely on 2-OH, but the assignment is not certain. Metabolite 6 is identified as a monosulfate conjugate of 3-butoxy-2-hydroxy-1-propanol.

Metabolite 11 is a glucuronide conjugate, and its molecular mass of 324 is equivalent to BGE + H2O + glucuronide. NMR showed several protons on carbons attached to O, which is in agreement with a diol structure and conjugation with a glucuronide. Metabolite 7 is identified as a monoglucuronide conjugate of 3-butoxy-2-hydroxy-1-propanol with the conjugation on either 1-OH or 2-OH.
proposed structure. Metabolite 9 is identified as O-butyl-N-acetylseryl.

The molecular mass (132) of metabolite 10 is consistent with that of a previously identified BGE metabolite, butoxyacetic acid (Eadsforth et al., 1985). The NMR data are consistent with the proposed structure. Metabolite 10 is identified as butoxyacetic acid.

Metabolite 11 has a molecular mass of 193, equivalent to BGE + N-acetylcysteine. The MS fragmentation is consistent with 3-butoxy-2-hydroxy-1-propanethiol and 2-acetylaminoacrylic acid being parts of the molecule. NMR shows two sets of CH$_3$ groups as AB quartets with chemical shifts around 3 ppm, consistent with two CH$_2$ groups attached to S; therefore, the attachment of the mercapturic acid is at the C-1 position. Metabolite 11 is identified as 3-butoxy-1-(N-acetylcystein-S-yl)-2-propanol.

Metabolite 12 did not ionize on ESI/MS analysis. NMR showed three CH$_3$O as triplets, implying that the metabolite might be 2-butoxyethanol. NMR of authentic 2-butoxyethanol showed an identical spectrum as that of metabolite 12. Metabolite 12 is identified as 2-butoxyethanol.

Metabolite 13 did not ionize on ESI/MS analysis. We were not able to identify this metabolite based on its NMR data, so metabolite 13 remains unidentified at this point.

**Identification of Urinary Metabolites in Mice.** HPLC analysis of urine of female mice administered a 200-mg/kg p.o. dose revealed metabolites with similar retention times as those present in rat urine (metabolites 1–13) and three additional radiolabeled peaks (metabolites 14–16) (Fig. 1B). The metabolite profile shown in Fig. 1B adequately describes the urinary metabolite profiles from female mice dosed with 20 mg/kg and male mice dosed with 200 mg/kg. Metabolite 14 had a similar HPLC retention time (17.7 min) as that of the aglycone of metabolite 7 after β-glucuronidase hydrolysis. Microsomal incubation (no NADPH) of BGE also yielded a sole product that had a similar retention time as that of metabolite 14, likely a diol from hydration of BGE catalyzed by epoxide hydrolase (data not shown). Metabolite 14 is identified as 3-butoxy-2-hydroxy-1-propanol. Female mouse urine was also analyzed by LC/ESI(−)/MS to reveal metabolites 2 through 9, 11, 15, and 16. Metabolite 15 has a molecular mass of 252, consistent with an α-hydroxy acid generated from oxidative deamination of a cysteine conjugate of BGE, 3-butoxy-1-(cystein-S-yl)-2-propanol (molecular mass, 251), followed by reduction. The MS fragmentation is consistent with 3-butoxy-2-hydroxy-1-propanethiol and 2-hydroxypropionic acid being parts of the molecule (Fig. 2). Metabolite 16 has a molecular mass of 250, consistent with an α-keto acid generated from oxidative deamination of the cysteine conjugate of BGE, 3-butoxy-1-(cystein-S-yl)-2-propanoic acid (molecular mass, 251). The MS fragmentation is consistent with 3-butoxy-2-hydroxy-1-propanethiol being a part of the molecule (Fig. 2).

The radioactivity in each of the peaks in the urine metabolite profiles from rats and mice was quantified as a percentage of the total radioactivity in all the peaks. The metabolites are products of two pathways, with the initial metabolism being either hydrolysis or GSH addition. Metabolites 1 through 3, 6 through 10, 12, and 14 are hydrolysis pathway products. Metabolites 4, 5, 11, 15, and 16 arise from an initial addition of GSH. Table 3 presents the result of the total percentages of metabolites in each of the two pathways.

**Incubation of BGE with GSH and GST.** The NMR and MS data of the sole product in the incubation show a GSH conjugate of BGE. Two sets of AB quartets with chemical shifts around 3 ppm are consistent with two CH$_2$ groups attached to S, indicating the addition of GSH occurred at the C-1 position of BGE. The product is identified as 3-butoxy-1-(glutathion-S-yl)-2-propanol. The product exists as two diastereomers with similar but not exactly the same NMR spectra. Therefore, the resonances from the two CH$_2$ groups attached to S are not well defined because of the overlapping of the signals from two diastereomers.

**Discussion**

The disposition of BGE in male and female rats is similar to the previous study of BGE in male rats treated with a single 20-mg/kg p.o. dose (Eadsforth et al., 1985). Both this and the previous (Eadsforth et al., 1985) studies show that p.o. administration of BGE results in rapid absorption and metabolism with most of the dose excreted in urine within 24 h. Most of the dose given to mice was also excreted in urine. A major difference between rats and mice is that mice excreted more of the dose in expired air as 14CO$_2$ (10–18%, 2–200 mg/kg) than rats (1.5%, 20 mg/kg). Less than 5% of the dose remained in the tissues after 24 h. After 10 consecutive daily 20-mg/kg doses, tissue concentrations in mmol-Eq BGE/g tissue were generally 2 to 4 times higher than after one dose (data not shown), indicating that there would be some accumulation of BGE and/or its metabolites in tissues during a chronic toxicity study. The highest concentration of BGE-derived radioactivity is in the stomach. This is also the only tissue where the concentrations may not be dose-proportional. It is a small tissue and the site of application, so nonlinearity is not unexpected.

Fifteen urinary metabolites of BGE were identified (Fig. 2). The metabolism of BGE follows two major pathways: 1) hydration to give a diol (metabolite 14), and 2) conjugation with GSH. Diol formation is followed by further metabolism to sulfate (metabolite 6) or glucuronide (metabolite 7) conjugates or an α-hydroxy acid (metabolite 8). Oxidation of the hydroxy acid to an α-keto acid provides a precursor for oxidative decarboxylation to butoxyacetaldehyde or transamination followed by acetylation to O-butyl-N-acetylseryl (metabolite 9). Butoxyacetaldehyde is further oxidized to butoxyacetic acid (metabolite 10) or reduced to 2-butoxyethanol (metabolite 12).

The structural identification of the GSH conjugate of BGE from the in vitro experiment and the urinary mercapturic acid metabolites 4, 5, and 11 indicates that GSH conjugation with BGE occurred only at the C-1 position. Metabolites 8, 9, 11, and 14 undergo further α-oxidation to form the corresponding 3-hydroxybutydoxy-substituted metabolites 2, 3, 4, and 1. Metabolite 5 is generated from α-oxidation of metabolite 11 to the corresponding carboxylic acid. The precursor to metabolite 11, 3-butoxy-1-(cystein-S-yl)-2-propanol, undergoes oxidative deamination in mice only to the corresponding α-keto acid 16, which is further reduced to an α-hydroxy acid 15. Some of the BGE metabolic pathways are common biosynthetic pathways for amino acids and fatty acids because of metabolism of BGE to an α-hydroxy acid and the long carbon chain in the structure.
Metabolites 7 and 14 were not totally separated by HPLC and therefore were quantified together, although the diol exists predominantly as an aglycone (metabolite 14) in mice and as a glucurononide conjugate (metabolite 7) in rats. Transamination and acetylation of the α-keto acid from oxidation of metabolite 14 to metabolite 9 is a major pathway in rats but not in mice, and as a result, the alternative metabolic pathway to metabolites 10 and 12 becomes dominant in mice, which may explain a larger amount of the dose excreted as CO₂ in mice. In comparison, oxidative deamination of 3-butoxy-1-(cysteine-S-yl)-2-propanol to metabolites 15 and 16 occurs in mice only, especially in female mice. The ratio of metabolites formed via the hydrolysis pathway compared with the GSH pathway is dose-related, at least in male rats (Table 3). A linear decrease in GSH pathway products as the dose increases implies a dose-related GSH depletion. The hydrolysis/GSH ratio is similar for rats and male mice given a dose of 200 mg/kg. Female mice appear to require a higher dose to deplete GSH sufficiently to change the hydrolysis/GSH ratio (Table 3). The dose-related GSH depletion may explain that more of the higher dose (200 mg/kg) excreted in expired air (14%, male mice and 18%, female mice) than the lower dose treatment (11%, 2 mg/kg, female mice and 10%, 20 mg/kg, female mice).

BGE is reported to cause central nervous system toxicity after acute i.p. or intragastric administration or inhalation exposure, but little BGE-derived radioactivity was detected in brain in this study. The epoxy group in BGE is reactive and may be responsible for the high forestomach/glandular concentration ratio, 4 to 5 in rats and 10 in mice, 24 h after a 200-mg/kg dose of BGE (Table 2). 2-Butoxyethanol is structurally similar to BGE but does not have an epoxy group and is not a direct alkylating agent. Nevertheless 48 h after a 500-mg/kg dose of 2-butoxyethanol to male rats, the forestomach contains relatively high concentrations of 2-butoxyethanol-derived radioactivity, about 4 times the concentration found in the adjacent glandular stomach (Ghanayem et al., 1987). Forestomach is a target tissue in the 2-year bioassay of 2-butoxyethanol in mice (NTP, 2000). Another target tissue associated with 2-butoxyethanol is erythrocytes. A metabolite, butoxyacetic acid, has been implicated in the erythrototoxicity (Ghanayem et al., 1989). Butoxyacetic acid and 2-butoxyethanol are BGE metabolites. Blood/plasma ratios for BGE-treated animals are about 1, indicating no increased uptake of BGE radioactivity by erythrocytes. At the doses given in this study no erythrototoxicity was observed. It is likely that BGE will cause site-of-application toxicity in a chronic study. Small amounts of butoxyacetic acid and 2-butoxyethanol from BGE metabolism may not cause any significant toxicity.

References

Address correspondence to: Ling-Jen Chen Ferguson, Lovelace Respiratory Research Institute, 2425 Ridgecrest Drive SE, Albuquerque, NM 87108. E-mail: ljferguson@lri.org