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Authors

Watson, AtLee TD
Moeller, Benjamin C
Doyle-Eisele, Melanie
et al.

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Disposition and metabolism of ethylene glycol 2-ethylhexyl ether in Sprague Dawley rats, B6C3F1/N mice, and *in vitro* in rat hepatocytes

AtLee T. D. Watson[1,2], **Benjamin C. Moeller**[3], **Melanie Doyle-Eisele**[3], **C. Edwin Garner**[3], **Chad R. Blystone**[1], **Jacob D. McDonald**[3], **Suramya Waidyanatha**[1,*]

[1]Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA;

[2]Integrated Laboratory Systems, Research Triangle Park, NC, USA (current affiliation);

[3]Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM, USA.

Abstract

1. Ethylene glycol 2-ethylhexyl ether (EGEHE) is a solvent used in a variety of applications.
2. We report disposition and metabolism of EGEHE following a single gavage or dermal administration of 50, 150 or 500 mg/kg [^{14}C]EGEHE in rats and mice and in vitro in rat hepatocytes.
3. EGEHE was cleared rapidly in rat hepatocytes (half-life ~ 4 min) with no sex difference.
4. EGEHE was well- and moderately absorbed following oral administration (rats: 80–96%, mice: 91–95%) and dermal application (rats: 25–37%, mice: 22–24%), respectively, and rapidly excreted in urine.
5. [^{14}C]EGEHE-derived radioactivity was distributed to tissues (oral: 2.3–7.2%, dermal: 0.7–2.2%) with liver and kidney containing the highest levels in both species.
6. EGEHE was extensively metabolized with little to no parent detected in urine. The alkoxyacetic acid metabolite, which has previously been shown to mediate toxicities of other shorter-chain ethylene glycol ethers, was not detected.
7. There were no apparent dose, species or sex differences in disposition and metabolism of EGEHE, except that the exhaled volatile compounds were greater in mice (19–20%) compared with rats (<2%).
8. These studies address a critical gap in the scientific literature and provide data that will inform future studies designed to evaluate toxicity of EGEHE.

*Correspondence: Suramya Waidyanatha, Division of the National Toxicology Program, National Institutes of Environmental Health Sciences, P.O. Box 12233, Mail Drop K2-07, Research Triangle Park, North Carolina 27709, waidyanathas@niehs.nih.gov.

Declaration of Interest

The authors have no real or perceived conflicts of interest to disclose.

Keywords

Ethylene Glycol 2-ethylhexyl Ether; EGEHE; absorption; distribution; metabolism; excretion; glycol ether; alkoxyacetic acid

Introduction

Ethylene glycol 2-ethylhexyl ether (EGEHE) belongs to a class of ethylene glycol ether (EGE) solvents used in several industrial and household products including paints, coatings and sealants, inks, bleaches and detergents, cosmetics, and personal care products (NTP, 2008). Human exposure to EGEHE occurs primarily via dermal contact; however, inhalation exposure may also occur due to its volatility, although to a lesser degree. EGEHE production and use has increased in recent decades, to between 1–10,000 million pounds per year (USEPA 2002), as it has begun to replace EGEs containing short alkyl chains (4 or less carbons) based on evidence of toxicity from animal studies and potential health concerns related to known human exposures.

Several short monoalkyl sidechain EGEs, including ethylene glycol monomethyl ether (EGME), ethylene glycol monoethyl ether (EGEE), and ethylene glycol monobutyl ether (EGBE), have been evaluated in subchronic repeat-dose toxicity studies which demonstrate a range of hematological (Bartnik et al., 1987; Ghanayem et al., 2000; Hong et al., 1988), immune (Exon et al., 1991; Smialowicz et al., 1991), embryo-fetal (Hanley et al., 1984; Hardin et al., 1982; Horton et al., 1985; Nagano et al., 1981; Nelson et al., 1984), and testicular (Chapin et al., 1985; Foster et al., 1987, 1984) toxicity in mouse and rat species. In addition, the National Toxicology Program (NTP) compared the toxicity profiles of EGME, EGEE, and EGBE following exposure for 13 weeks via drinking water. In these studies, adult rats were generally more sensitive to the effects of EGE exposure than were adult mice, and toxicity was generally greater for shorter alkyl sidechain lengths (EGME > EGEE > EGBE) (NTP, 1993). However, despite widespread use and potential for exposure, there are limited toxicity data for EGEHE. Acute studies involving a single dose of EGEHE result in low to moderate toxicity when administered via dermal (rabbit LD_{50} = 2584 mg/kg) or oral (rat LD_{50} = 3080–7832 mg/kg; mouse LD_{50} = 3890–7308 mg/kg) routes (Eastman Kodak, 1992; NTP, 2008). Given the lack of toxicity data for EGEHE and its structural similarity to other EGEs, NTP is testing the potential toxicity of EGEHE in rodent models following oral exposure (NTP Testing Status for EGEHE).

Absorption, distribution, metabolism, and excretion (ADME) data are integral to the design of toxicity studies and interpretation of study data. To the best of our knowledge, there are no ADME data in the literature for EGEHE. Here we report findings from ADME studies following gavage administration and dermal application in Hsd:Sprague Dawley®SD® (HSD) rats and B6C3F1/N mice, two rodent models commonly used in NTP studies.

Materials and methods

Chemicals and reagents.

Ethylene glycol 2-ethylhexyl ether (EGEHE) was obtained from Sigma Aldrich (CASRN 1559-35-9; Lot MKBP338V; St Louis, MO). Identity of EGEHE was confirmed by ^{1}H and ^{13}C NMR and mass spectrometry (MS) and the purity (>99.9%) was determined by gas chromatography (GC) with flame ionization detection. [^{14}C]-labeled EGEHE ([^{14}C]EGEHE); 12.1 mCi, 0.19 mCi/mg in ethanol) (Figure 1) was obtained from Moravek Biochemicals (Lot 265-161-05810A20140619-DRE; Kansas City, MO) and was stored at −20°C. The radiochemical purity was determined to be >99% by high performance liquid chromatography (HPLC) Method 1 described below.

Ethylene glycol butyl ether-d_4 (EGBE-d_4, internal standard) was purchased from C/D/N isotopes (Point-Claire, Quebec). 2-[(2-ethylhexl)oxy] acetic acid (2-EHOA) was synthesized by Tractus Chemical Company (London, UK). Corn oil, Trypan Blue solution, 2-hexyloxy-acetic acid (2-HOA), methoxyacetic acid (MOA), 7-ethoxycoumarin, β-Glucuronidase (from *E. coli*), sulfatase (from *Aerobacter aerogenes*), and acylase (Type I, porcine kidney) were purchased from Sigma-Aldrich. Ultima Gold™ and Ultima Gold XR liquid scintillation cocktail were purchased from PerkinElmer (Waltham, MA). All other reagents were standard laboratory grade or better and were obtained from commercial sources.

For the in vitro experiments, ethylene glycol butyl ether (EGBE) was obtained from Sigma-Aldrich (CASRN 112-25-4; Lot SHBD6844V) and ethylene glycol (mono) *n*-hexyl ether (EGHE) was obtained from Tokyo Chemical Industry (TCI; CASRN 112-25-4; Lot BQNM; Portland, OR). Cryopreserved adult (8- to 10-weekold) Sprague Dawley rat hepatocytes [lot 031–025 (male), lot 031–027 (female)] and Universal Cryopreserved Recovery Medium were purchased from IVAL (Columbia, MD). Phenol red free Dulbecco's Modified Eagle's Medium was obtained from Invitrogen (Carlsbad, CA).

Study Design.

The study design is given in Table 1. The effect of dose in ADME was investigated in male rats following a single gavage administration of 50, 150, and 500 mg/kg. The highest and lowest doses selected were approximately 0.1X and 0.01X of oral LD_{50} in rodents (Eastman Kodak, 1992). Sex and species differences were investigated in a set of limited studies following a single gavage administration of a 50 mg/kg dose in female rats and male and female mice. Dermal studies were also conducted in male and female rats and mice following application of a single 50 mg/kg dose to a covered and uncovered (male rats only) dose site. In addition, comparative clearance and metabolism of EGEHE with EGHE and EGBE in male or female rat hepatocytes were investigated.

HPLC Method 1.

HPLC Method 1 was used to determine purity of [^{14}C]EGEHE lot and dose formulations. The HPLC instrumentation consisted of an Agilent 1100 HPLC system (Agilent Technologies; Santa Clara, CA) which included a quaternary pump, a degasser, a column heater, an autosampler, and a variable wavelength detector coupled to a β-RAM-LS detector

with a 75-μL lithium glass, flow-through, solid flow cell (IN/US, Tampa, FL). A Phenomenex MAX-RP column (2.1 × 100 mm, 2.5 μm particle size; Torrance, CA) was used for analyte separation. Mobile phases A (0.2% aqueous acetic acid), B (0.2% acetic acid in acetonitrile) and C (50:40:10 acetonitrile:isopropyl alcohol:acetone) were run at a flow rate of 0.5 mL/min. Initial conditions were 98% A:2% B held for 1 min, followed by a linear gradient to 2% A:98% B over 20 min, held for 2 min and followed by 100% C for 0.9 min.

HPLC Method 2.

HPLC Method 2 was used to profile urine before and after deconjugation experiments. The system used was the same as in HPLC Method 1. A Phenomenex Hydro-RP column (4.6 × 5 mm, 4 μm particle size; Torrance, CA) was used for analyte separation. Mobile phases A (10 mM ammonium acetate, 0.015% formic acid in water) and B (10 mM ammonium acetate, 0.015% formic acid in 45:45:10 acetonitrile:methanol:water) were run at a flow rate of 1.0 mL/min using initial conditions 95% A held for 1 min, followed by a linear gradient to 55% A over 20 min.

LC-MS Method 1.

Liquid chromatography-mass spectrometry (LC-MS) Method 1 was used to quantitate clearance of EGEHE, EGHE, EGBE, and EGBE-d4 (internal standard) in vitro using rat hepatocytes. The LC-MS instrumentation included an Agilent 1200 HPLC (Agilent, Santa Clara, CA) coupled to an API 5000 (Applied Biosystems, Foster City, CA) mass spectrometer, equipped with a TurboIon electrospray ionization source operating in positive ionization mode. Source temperature and ion spray voltage were 450 °C and 5500 V, respectively. Chromatography was performed using a Mercury MS Synergi MAX-RP column (2 × 100 mm, 2.5 μm particle size) (Phenomenex, Torrance, CA) maintained at 50 °C. Mobile phase A (0.2% aqueous acetic acid), B (0.2% acetic acid in methanol) and C (50:40:10 acetonitrile:isopropyl alcohol:acetone) were run at a flow rate of 0.25 mL/min using initial conditions 80% A:20% B held for 1 min followed by a linear gradient to 2% A:98% B over 9 min, and held for 3 min before changing to 100% C for 1 min. The transition monitored for quantitation of EGEHE was m/z 175.1→ 113.1, 63.0; EGBE m/z 118.8 → 62.0, 56.7; EGHE m/z 147.2 → 85,0, 44.6; and EGBE-d4 m/z 123.2 → 56.7, 66.7.

LC-MS Method 2.

LC-MS Method 2 was used to analyze putative alkoxyacetic acid metabolites of EGEHE. An Agilent 1100 HPLC (Santa Clara, CA) coupled with a PE Sciex (Concord, Ontario) API 365 with an EP10 upgrade triple stage quadrupole mass spectrometer was used with a Phenomenex Hydro-RP column (4.6 × 50, 4 μm) (Phenomenex, Torrance, CA). Mobile phase A consisted of 10 mM ammonium acetate, 0.0125% formic acid in water; mobile phase B was 10 mM ammonium acetate, 0.015% formic acid in 45:45:10 acetonitrile:methanol:water run at a flow rate of 1.0 mL. Initial conditions were 95% A:5% B for 1 min, followed by a linear gradient to 55% A:45% B over 20 min, and to 2% A:98% B over 3 min and was held for 2 min. UV scans were acquired from 190–400 nm on an Agilent diode array detector. Data were acquired using positive and negative mode

electrospray ionization with Q1 full scan MS (m/z 40–250) and product ion scans. The ion source temperature was 300 °C and spray voltage was 4200 V.

LC-MS Method 3.

LC-MS Method 3 was used to screen urine samples for potential metabolites. LC-MS instrumentation consisted of an Agilent 1100 HPLC (Santa Clara, CA) coupled with a PE Sciex API 365 (Concord, Ontario) with an EP10 upgrade triple stage quadrupole mass spectrometer. Mobile phase A consisted of 10 mM ammonium acetate, 0.015% formic acid in water; mobile phase B was 10 mM ammonium acetate, 0.015% formic acid in 45:45:10 acetonitrile/methanol/water. A gradient was used with a flow rate of 1.0 mL/min using a Phenomenex Hydro-RP column (4.6 × 5 mm, 4 μm particle size) (Phenomenex, Torrance, CA). Initial conditions were 95% A:5% B for 1 min, followed by a linear gradient to 55% A/45% B over 20 min and to 2% A/98% B over 3 min, which was held for 2 min. The mass spectrometer was operated in positive and negative ESI modes and MRM scans, Q1 full scan MS, constant neutral loss (m/z 80, 129, 179), and precursor ion scans. The ion source temperature was 300 °C and voltage was 4200 V. Predicted MRM transitions are given in Table S1.

GC-MS Method.

GC-MS data for 2-EHOA were acquired on an Agilent 6890 (Agilent, Santa Clara, CA)) coupled to a 5973N Mass Selective Detector (MSD). An RTX-5MS column (30 m, 0.25 mm ID, 0.25 μm df) (Restek Corp; Bellefonte, PA) with helium flow rate of 1.6 mL/min. The injector temperature was 200 °C. The oven temperature was maintained at 75°C for 10 min, followed by ramp of 6°C/min up to 120°C, followed by 15°C/min to 250°C that was held for 5 min. The mass spectrometer transfer line, ion source, and quadrupole temperatures were set to 280°C, 230°C, and 150°C, respectively. The mass spectrometer was scanned from m/z 20 to 300. 2-EHOA eluted with a retention time of 19.1 min.

In vitro studies.

Clearance of EGEHE, EGBE, and EGHE was investigated in cryopreserved Sprague Dawley rat hepatocytes. Hepatocyte suspensions are commonly used to investigate hepatic clearance and metabolism (Wetmore et al 2012). Cells were thawed and resuspended according to the supplier's protocol. Viability was assessed by Trypan blue exclusion. The cell concentrations were 1×10^6 cells/mL and viabilities were >85%.

Stock solutions of test articles were prepared in methanol and diluted with water to prepare 10 μM solution (final methanol concentration in spiking solution was <1%). Incubations were performed in triplicate using polypropylene round bottom 24-well cell culture plates (Becton Dickinson, East Rutherford, NJ) at a volume of 0.5 mL/well. The incubation media consisted of phenol-free Dulbecco's Modified Eagle's Medium. Each well consisted of approximately 0.5×10^6 cells with a final test article concentration of 1 μM. Controls consisted of heat deactivated hepatocytes (100 °C, 10 min) with test article, hepatocytes with 7-ethoxycoumarin (20 μM, positive control), and media only with test article, and PBS (pH 7.2). Plates were incubated at 37°C with a 5% CO_2 atmosphere and with shaking. Each assay was initiated by the addition of 50 μL of 10 μM glycol ether solution. Aliquots (50 μL)

of the suspension were taken at 0 (prior to addition of test article), 5, 10, 30, 60, and 120 min after initiation of the experiment. Aliquots were added to a 1.5 mL tube containing 100 μL acetonitrile with 0.5 μM EGEBE-d4 as the internal standard. Samples were stored at −20 °C until analysis, at which point samples were thawed and centrifuged at 13,000 × *g* for 5 min and supernatants were removed for analysis.

Six-point calibration curves (0.1 to 2 μM) and quality control (QC) samples (0.75, 1.25 and 1.8 μM) were prepared similar to samples above but using incubation medium. All samples and standards were analyzed using LC-MS Method 1 for analyte quantitation. Calibration curves relating peak area ratios of test article to internal standard (EGBE-d4) as a function of analyte concentration were constructed using regression model with $1/x^2$ weighting. The linearity (>0.99), accuracy (determined as percent relative error, ≤ ±15%), precision (determined as percent relative standard deviation, ≤15%)) and recovery (32–75%) demonstrated that the methods were suitable for quantitation of the test articles in hepatocyte incubation samples. The concentration of test article in samples was calculated using the response ratio of the test article to internal standard and the regression equation.

Using the natural log of concentration versus time data, half-lives of elimination ($t_{½}$) were estimated. Intrinsic clearance (Cl_{int}, mL/(min/kg body wt.)) was calculated using the equation: Cl_{int} = 0.693/t1/2 * mL incubation/cells * 120 million cells/g liver * g liver/kg body wt. using the physiological parameters for liver weight of 34 g/kg body wt. for rat (Brown et al., 1997; Sohlenius-Sternbeck, 2006).

Animals.

Studies were conducted at Lovelace Biomedical Environmental Research Institute (LBERI) (Albuquerque, NM) and were approved by Institutional Animal Care and Use Committee. Animals were housed in facilities that are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Animal procedures were performed in accordance with the “Guide for the Care and Use of Laboratory Animals” (NRC, 2011). Male and female Hsd:Sprague Dawley®SD® (HSD) rats, including the animals with closed loop bile catheters, were obtained from Harlan Laboratories (Livermore, CA). B6C3F1/N mice were obtained from Taconic Farms (Germantown, NY). Animals were quarantined for approximately two weeks, except bile duct-cannulated HSD rats which were held in quarantine for one week, prior to randomization into dosing groups. All animals had *ad libitum* access to certified, irradiated NTP 2000 feed (Ziegler Bros, Inc., Gardners, PA) and municipal (Albuquerque, NM) tap water. Environmental conditions included: room temperature of 72 ± 7°F (22 ± 4 °C), relative humidity of 30 to 65%, and a 12-h light/dark cycle. Animals were acclimated for 24 h in individual metabolism cages prior to dosing and were returned to cages following dosing to allow for separate collection of excreta. Animals were 8–10 weeks old at the time of dosing. At the end of each study, animals were anesthetized by inhalation of CO_2:O_2 (70:30), blood was collected via by cardiac puncture, and animals were subsequently euthanized by section of the diaphragm.

Formulation preparation and administration of EGEHE.

All dose formulations were prepared the day prior to administration and contained [^{14}C]EGEHE and unlabeled EGEHE to achieve the final desired EGEHE concentration and specific activity. The target radioactivity per animal was approximately 50 μCi/rat and 10 μCi/mouse. Oral dose formulations were prepared in corn oil with target doses of 50, 150 and 500 mg/kg; a single dose was administered in a volume of 5 mL/kg for rats and 10 mL/kg for mice by intragastric gavage. Dermal formulations were prepared in ethanol with a target dose of 50 mg/kg. On the day prior to dermal application, animals were anesthetized and the hair from the intrascapular region was clipped from an area no less than 4 cm^2 and cleaned with soap and water and the dose site was marked (1 cm^2). For animals in the covered dermal studies, the metal dermal applicator (Shandon Lipshaw Microcassette #331 positive clip, LabX Scientific Marketplace) was applied to the dose site with cyanoacrylate glue (Krazy Glue; Westerville, OH). Dermal applications were applied at a volume of 0.5 mL/kg for rats and 2 mL/kg for mice using a 250- or 500-μL Luer lock gas-tight syringe (Hamilton; Reno, NV). For all studies, dosing volumes were determined based on individual animal weights. The concentration of [^{14}C]EGEHE in each dose formulation was determined by liquid scintillation spectroscopy (LSS) using a Packard Model 3100TR liquid scintillation analyzer of three aliquots collected pre- and post-dosing. Radiochemical purity of each formulation was assessed using HPLC Method 1 on the day of dosing and was ≥ 99%.

Collection and analysis of biological samples.

Samples were collected and analyzed as described previously (Mutlu et al., 2019; Waidyanatha et al., 2020b). All samples were stored at −20 °C until analysis. Briefly, urine and cage rinse were collected from each animal at the following intervals: 0–8 h, 8–24 h, and 24 h intervals as appropriate. Feces were collected at 0–24 h and 24 h intervals as appropriate. Urine and feces were cooled on dry ice. At the end of each collection interval, the cage was rinsed with water and ethanol and the rinsate was collected and stored. Bile was collected from bile duct cannulated animals over the 0–4, 4–8, 8–12, and 12–24 h intervals. Volatile organic compounds (VOC) and CO_2 were collected at the following intervals: 0–4, 4–8, 8–12, 12–24, 24–48, and 48–72 h intervals. Urine, feces, and air (from CO_2 and VOC traps) were collected during the acclimation period to determine radioactivity from metabolism cages. At the termination of each study group, following euthanasia, blood, tissues, and excreta were collected as described previously (Mutlu et al. 2019; Waidyanatha et al. 2020). In bile duct-cannulated animals, bile that was in the line was drained into the vial for the last collection time point. Samples were analyzed for radioactivity content by LSS with external standards quench correction.

For determination of total [^{14}C]EGEHE in dispersed tissues, the total rat weight was assumed to be comprised of 7.4% blood, 4.3% plasma, 7.0% adipose, 40.4% muscle, and 19% skin, and the total mouse weight was assumed to be comprised of 4.9% blood, 2.8% plasma, 7.0% adipose, 38.4% muscle, and 16.5% skin (Brown et al., 1997).

Metabolite profiling and identification.

Urine pools were created per collection period and dose group by combining an equal volume of each animal's urine collection time period and group, when greater than 1% of the radioactivity was recovered in urine. Urine pools were diluted 1:1 with methanol:water and analyzed before and after deconjugation with β-glucuronidase, sulfatase, and acylase. β-glucuronidase (25,000 U/mL) was prepared in 0.1 M phosphate buffer (pH 6.8). Sulfatase (50 U/mL) and acylase I (20,000 U/mL) were prepared in 0.2 M ammonium acetate (pH 5.0 sulfatase; pH 7.0 acylase I). In deconjugation experiments, 150 μL of pooled urine samples were combined with 20 μL of enzyme, and 30 μL of either 1 M sodium phosphate (β-glucuronidase), 2 M ammonium acetate pH 5.0 (sulfatase), or 2 M sodium phosphate pH 7.0 (acylase I). Samples were incubated at 37 °C for 1 h (β-glucuronidase and sulfatase) or 2 h (acylase I). Reactions were stopped by adding 60 μL of chilled ethanol followed by addition of 50 μL water. Samples were centrifuged for 5 min at 14,000 × *g* and supernatants were collected. Samples were analyzed using HPLC Method 2 and LC-MS Method 3. As an initial assessment to determine presence/absence of 2-EHOA, pooled urine was extracted with dichloromethane prior to analysis by GC-MS described above. Standards prepared in urine at the 10 μg/mL were run along with the samples to ensure performance of the instrument.

Results

The data for individual animal study groups are available in the NTP Chemical Effects in Biological Systems (CEBS) database and summaries are provided in the manuscript: https://manticore.niehs.nih.gov/cebssearch/paper/14789

In vitro studies.

Clearance of EGEHE, EGHE and EGBE (males only) was investigated in male and female rat hepatocytes at 1 μM and data are shown in Table 2. In general, in male rat hepatocytes, clearance increased with the length and size of the side chain of the glycol ether with 669, 62.1, and 35.6 mL/min/kg for EGEHE, EGHE, and EGBE, respectively. There was no sex difference in clearance for EGEGE (females 645 mL/min/kg), however, for EGHE (females 161 mL/min/kg) a sex difference in clearance was observed. The resulting half-lives were 4.39 and 4.32 min for EGEHE and 45.5 and 17.6 min for EGHE in male and female hepatocytes, respectively. Lastly, male hepatocytes incubated with 1 μM EGBE showed the longest half-life of 79 min.

Disposition of [^{14}C]EGEHE in rats.

The disposition of radioactivity up to 72 h following a single gavage administration in male (50, 150, or 500 mg/kg) and female (50 mg/kg) rats is shown in Table 3. In male rats administered 500 mg/kg [^{14}C]EGEHE and followed through 72 h after administration, excretion was complete by 48 h and hence, in subsequent groups the study duration was set at 48 h (except the tissue distribution study which was up to 24 h). In male rats, [^{14}C]EGEHE was well-absorbed following gavage administration with 54–73% of the administered dose recovered in urine (including cage rinse) and 13–17% excreted in the feces. Excretion was rapid with the majority of the dose recovered in urine and feces within

the first 24 h. Exhaled VOC contained trace amounts of radioactivity and low amounts (2–4%) were detected in exhaled CO_2 (Table 3). In male rats administered 50 mg/kg [^{14}C]EGEHE, trace levels of radioactivity (<0.05% of the administered dose) were found in 24-h bile suggesting that dose recovered in feces following oral administration is the unabsorbed dose and not the absorbed dose excreted via bile into the intestine. Comparison of data from male and female rats administered 50 mg/kg and sacrificed at 48 h after administration found similar excretion profiles of [^{14}C]EGEHE (males: 69% urine, 17% feces; females: 68% urine, 11% feces) (Table 3). In addition, there did not appear to be dose-related changes in the percentage of recovered dose in excreta between the 50, 150, and 500 mg/kg dose in male rats.

[^{14}C]EGEHE was distributed to all tissues investigated with concentrations increasing with the dose in male rats; the total administered dose recovered by 48 h (72 h for 500 mg/kg) was between 4.6 and 5.9% (Tables 3 and 4). Following administration of 50 mg/kg in males the concentration of radioactivity in tissues was higher at 24 h than at 48 h with 7.2% and 4.6% of the administered dose recovered, respectively, suggesting elimination of radioactivity in tissues with time. In females, total radioactivity in tissues at 48 h was slightly lower (2.3%) than 50 mg/kg males at 48 h (Table 4). The tissue-to-blood ratios (TBRs), in general, were low with the highest value found in the liver (2.1–5.4), kidney (1.0–2.3), spleen (1.2–2.5), and thyroid (1.1–2.9). Higher TBRs observed for bladder and gastrointestinal (GI) tract tissues were likely due to contamination from urine and feces, respectively. The total administered dose recovered following oral administration in male and female rats ranged from 80–96% (Table 3).

Disposition was also assessed following dermal application of 50 mg/kg [^{14}C]EGEHE to an uncovered (males only) and covered dose site in males and females (Table 5). Following application to a covered site, 29–31% and ~ 3% of the applied dose was excreted in urine and feces at 72 h, respectively, with 1–2% found in CO_2 and tissues. By comparison, uncovered application resulted in similar excretion pattern with urine (including cage rinse) and feces containing approximately 19% and ~ 4% of the total applied dose, respectively. The dose recovered at the application site in all groups was ≤ 0.5%. For both uncovered and covered administration, VOC contained a significant percentage of the applied dose of [^{14}C]EGEHE (21–39%), all or part of which could be due to direct evaporation from the dose site. The majority of excreted [^{14}C]EGEHE occurred in the first 24 h after administration suggesting rapid dermal absorption. Based on these data, at least 36–37% of a dermally applied dose of [^{14}C]EGEHE was absorbed in male and female rats following application to a covered dose site (Table 5).

Tissue distribution of [^{14}C]EGEHE was 0.9–2.2% of the total administered dose (Table 5). Overall, distribution to individual tissues was similar between oral and dermal application, with the highest TBRs found in liver (3.5–4.1), kidneys (1.8–23.2), thyroid (1.7–7.5), and lungs (1.9–5.1) (Table 6). The total dose recovered following dermal application of [^{14}C]EGEHE in male and female rats to a covered dose site was 71–79%. The total dose recovered following dermal application to an uncovered site was poor (~ 46%) likely due to loss of [^{14}C]EGEHE from the dose site due to volatilization.

Disposition of [^{14}C]EGEHE in mice.

Disposition of radioactivity 48 h following oral administration of 50 mg/kg [^{14}C]EGEHE in male and female mice is shown in Table 7. Following a single gavage dose, [^{14}C]EGEHE was well absorbed with 27–48% excreted in the urine (including cage rinse) and 17–36% in feces. Higher radioactivity was found in feces from females (36%) than males (17%), likely attributed to fecal contamination with urine given the lower radioactivity found in females (27%) compared to males (48%). Excretion was rapid with the majority of [^{14}C]EGEHE recovered within 24 h. Exhaled VOC and CO_2 accounted for 19–20% and 4–6% of the total administered dose, respectively. Overall, excretion profiles were similar between male and female mice.

Approximately 4% of [^{14}C]EGEHE-derived radioactivity was present in tissues in males and females (Table 7) at 48 h following oral administration. [^{14}C]EGEHE was well distributed as radioactivity was detected in all tissues assayed. TBRs were generally higher in tissues associated with excretion and metabolism and included the liver (6.5–7.9), kidneys (2.8–3.5), and lungs (2.3–2.8). Similar to rats, the higher TBRs observed in the bladder and gastrointestinal tract were likely due to contamination with urine and feces, respectively (Table 8). The total administered dose recovered following oral administration in male and female mice ranged from 91–95%

Disposition was investigated following a 72-h covered dermal application of 50 mg/kg (Table 5). Excretion was similar between sexes with 13–14% of the total applied dose found in urine (including cage rinse) and 4–8% found in fecal matter at 72 h. Approximately 2% was detected in CO_2. The total dose recovered from the dose site was ≤ 2%. A significant percentage of radioactivity (54–58%) was detected in the VOC fraction, which could be due to a combination of exhaled VOC and direct evaporation from the dose site. The majority of the excreted radioactivity was recovered within the first 24 h, suggesting rapid dermal absorption. Based on these data, at least 22–24% of the covered dermal dose of [^{14}C]EGEHE was absorbed in male and female mice (Table 5).

Tissue distribution of [^{14}C]EGEHE-derived radioactivity was similar in males and females (~ 0.7%) following dermal application of 50 mg/kg (Table 5). Similar to gavage exposure, TBRs were consistently higher in the liver (4.7–5.1), kidneys (3.4–4.8), lungs (3.0–4.1), and thyroid (~ 4) than in other tissues (Table 8). Contamination from urine likely contributed to the higher TBRs observed in the bladder. The total recovery in mice following dermal application was 84 – 85%.

Profiling of [^{14}C]EGEHE metabolites in rats and mice.

A representative HPLC chromatogram for male rat urine following oral administration of 500 mg/kg [^{14}C]EGEHE is depicted in Figure 2A. Under these conditions parent EGEHE elutes ~ 24 min. Urinary chromatograms following oral administration showed a majority of peaks with retention times between 5–10 min and no parent was detected. Two peaks with retention times at ~ 7.9 and ~ 8.5 min accounted for more than 60% of the total peak area in the chromatogram.

To determine the presence of conjugated metabolites, pooled urine from male rats administered 500 mg/kg [^{14}C]EGEHE via gavage were incubated with β-glucuronidase, sulfatase, and acylase and were analyzed under similar conditions. Enzymatic digestion caused modest changes in the relative peak areas for several of the major metabolites. Treatment of urine with β-glucuronidase resulted in the disappearance of three minor peaks between 5.4 and 8 min and appearance of two minor peaks at 1.6 and 4.5 min (Figure 2B). The largest peak at ~8.5 min (55%, undigested) was reduced by 16, 23, and 29% following treatment with sulfatase, β-glucuronidase, and acylase, respectively, along with concomitant formation or slight increase in earlier peaks. For example, the second largest peak (~7.5 min) increased by 34, 19, and 84% with incubation in sulfatase, β-glucuronidase, and acylase, respectively (Figures 2B, 2C, 2D). Urinary radiochromatograms following dermal application of [^{14}C]EGEHE was similar to that following oral although fewer peaks were observed (chromatogram not shown). Urinary chromatograms in mice were similar to those of rats with minor differences; up to 16 peaks were detected including a small peak corresponding to parent [^{14}C]EGEHE in chromatograms following both gavage administration and dermal application (chromatograms not shown).

Urine from male rats orally administered 500 mg/kg [^{14}C]EGEHE was used for metabolite identification using LC-MS/MS with predicted MRM transitions (Table S1) and neutral loss experiments. A summary of corresponding data is provided in Table S2. Negative ion MRM mode identified peaks corresponding to potential hydrogenation, sulfate conjugation, or hydroxylation and methylation of the hexyl chain. In positive MRM mode, peaks corresponding to potential reduction and methylation (mono-, di- or tri-) or possibly acetylation transitions were observed at multiple retention times. In addition, peaks corresponding to conjugates of glucuronide (m/z 351 ➔ 71), glycine (m/z 232 ➔ 128), and sulfate (m/z 255 ➔ 151) were detected at ~ 18, ~ 15.5, and ~ 10 min, respectively. In constant neutral loss experiments, several peaks corresponding to the loss of a sulfate (m/z 80) were observed at ~ 2.1, 6, 7.2, 8, 9, and 10 min under positive ion mode (Figure 3A). The precursor ions for the sulfate metabolites were m/z 191, 205, 206, 207, and 208 indicating additional phase I modifications to parent EGEHE prior to phase II sulfation. In addition, under negative ion mode and neutral loss scan, single peaks were detected at ~ 10 min corresponding to the loss of a glucuronide (m/z 176) moiety (Figure 4A) and at ~ 7 min corresponding to a loss of a mercapurate (m/z 129) moiety (Figure 4B).

A targeted analysis was conducted to determine if EGEHE is metabolized to one or more alkoxyacetic acids, the toxic metabolites associated with shorter chain ethylene glycol ethers. Under these conditions, the retention times of 2-EHOA, 2-HOA, and MOA standards were 0.5, 12, and 19.8 min, respectively. Parent EGEHE eluted at ~ 23.7 min. However, under the same conditions, peaks matching those for 2-EHOA, 2-HOA, or MOA were not detected in urine from male rats administered [^{14}C]EGEHE (data not shown). Hence, a GC-MS method was developed for detection of 2-EHOA. Under these conditions the 2-EHOA standard eluted at 19.3 min with the spectrum showing ions of m/z 129, 111, 83, and 57 (Figures S1A and B). Although rat urine dosed with 500 mg/kg showed a peak at ~ 19.1 min, the mass spectrum did not match that of the 2-EHOA standard (Figure S1). Based on these data in male rats, EGEHE does not appear to be metabolized to the alkoxyacetic acid metabolites 2-EHOA, 2-HOA, or MOA. EGEHE appears to be extensively metabolized

through a number of phase I modifications followed by phase II sulfate conjugation, and glucuronide, glycine, and mercapurate conjugations to a lesser extent. However, definitive confirmation following isolation of individual metabolites were not conducted. Based on these analyses, proposed metabolic pathways of EGEHE in rodents are shown in Figure 5.

Discussion

Production and use of EGEHE in industrial and consumer products, namely paints, coatings, and sealants, has increased in recent decades as ethylene glycol ethers (EGEs) with shorter alkyl sidechains (e.g., EGME, EGEE, and EGBE) are being phased out over reported toxicity concerns. Therefore, the primary objective for this study was to elucidate ADME parameters to better inform subsequent studies aimed at evaluating the toxicological potential for EGEHE. Exposures via oral and dermal routes were evaluated, the latter being the most likely route of exposure in humans in occupational settings.

Overall, [^{14}C]EGEHE was well absorbed following oral administration in rats (50–500 mg/kg) and mice (50 mg/kg). In rats, 65–81% of the total dose was absorbed, the majority of which was found in urine and within 24 h following dosing. A significant percentage (11–17%) of the total dose was recovered in feces. In bile-duct cannulated male rats little to no radioactivity was found in bile suggesting that fecal radioactivity likely represented unabsorbed [^{14}C]EGEHE dose. Absorption was similar in mice (54–77%) with the majority of the administered dose recovered in urine within 24 h. The lower estimated absorption in males (54%) was most likely attributed to contamination of fecal samples with urine evidenced by higher levels in feces (37% males, 17% females) along with concomitant lower levels observed in urine (27% males, 48% females), a not uncommon finding in disposition studies conducted in mice (Waidyanatha et al., 2020a). A notable difference between mice and rats was observed in the exhaled VOC fraction, which accounted for ~ 20% of the total dose in mice compared to less than 1% in rats at the same dose level (50 mg/kg), suggesting at least one or more potential differences in EGEHE metabolism and/or excretion between the two species.

Following gavage exposure, tissue distribution of [^{14}C]EGEHE-derived radioactivity was generally similar between rats and mice. In rats, the greatest tissue burden (7.1%) was identified in males at 24 h after exposure and decreased in dose groups terminated at 48 or 72 h, providing further evidence of rapid elimination of [^{14}C]EGEHE from tissues. For comparison, tissue distribution in male and female mice administered 50 mg/kg was ~ 4% for male and females. In both species, tissue distribution and retention was low as evidenced by low TBRs. Interestingly, slightly higher TBRs were observed at 48 h in the lungs of mice (2.3–2.8) compared to rats (0.9–1.5), providing additional context to support exhalation as an elimination route in mice following oral exposure. Our findings of rapid absorption and elimination, and low tissue burdens following oral administration suggest low bioaccumulation potential for EGEHE in rodents.

Similar findings have been reported in rats following oral exposure to other ethylene glycol mono-alkyl ethers (e.g. EGME, EGEE, EGBE, and EGHE). Following a single gavage exposure in rats, EGME, EGEE, and EGBE were rapidly absorbed and excreted in urine, the

majority of which was recovered within 24 hours (Cheever et al., 1984; Ghanayem et al., 1987b; Miller et al., 1983). A larger proportion of radiolabeled EGME (~ 12%), EGEE (5–12%), and EGBE (10–18%) were recovered in CO_2 compared to EGEHE, where less than 6% of [^{14}C]EGEHE was recovered in the CO_2 fraction (rats or mice). Ghanayem et al speculated that recovery of radiolabeled-EGEE in the CO_2 fraction could have resulted from metabolism of parent EGBE to butyric acid and subsequent entry into the fatty acid cycle where CO_2 is formed as a byproduct. In the same study, EGBE was distributed to all tissues, and the highest EGBE concentrations were found in the liver, kidney, and spleen (Ghanayem et al., 1987b). Additionally, a 24-hour drinking water exposure study in male F344 rats comparing disposition of EGME, EGEE, and EGBE observed rapid absorption and elimination for all three EGEs with 40–80% of total dose recovered in urine (Medinsky et al., 1990). Urinary excretion appeared to increase with increasing chain length (EGME<EGEE<EGBE) while excretion in the CO_2 fraction (10–30%) followed the opposite trend (EGME>EGEE>EGBE). It remains unclear why lower amounts of EGEHE were excreted via CO_2 relative to EGME, EGEE, EGBE, but it could represent a continuation of the trend observed by Medinsky et al (1990).

Following dermal application (50 mg/kg [^{14}C]EGEHE), EGEHE demonstrated moderate absorption in rats (25–37%) and mice (22–23%). For the present study, we did not include the VOC fraction when determining the percent of total dose absorbed. Given the volatility of glycol ethers, it is likely that a significant portion of the VOC fraction represents evaporation of the material from the application site. In both species, EGEHE was rapidly absorbed as the majority of the radioactivity in excreta (urine, feces, CO_2) was collected within the first 24 hours post application. Similar tissue distribution of EGEHE was noted between rats (1–2%) and mice (~ 0.7%) at 72 h, and there were no apparent sex differences in disposition following dermal application. The findings of rapid absorption and excretion in conjunction with low TBRs in most tissues suggest low potential for bioaccumulation of EGEHE following dermal application. One notable difference between mice and rats was the VOC fraction, which accounted for a significant percentage of the total dose in both species and was higher in mice (54–58%) compared to rats (21–39%). HPLC analysis of the VOC samples from rats and mice identified a single peak corresponding to parent [^{14}C]EGEHE. It is worth noting that exhaled VOCs accounted for 20% of the total dose in orally exposed mice, which could explain the two-fold greater recovery of [^{14}C]EGEHE in the VOC of dermally-exposed mice compared to rats. In addition, species differences in the rate of volatilization of compound from the dermal site may also have played a role in the observed differences. This may underestimate dermal absorption of EGEHE, especially for mice; however, the relative contributions of evaporation from the application site versus absorbed and exhaled [^{14}C]EGEHE cannot be ascertained under the conditions of the present study. The evaporation of the compound from the application site is further supported by the lower overall dose recovered in rats following application to an uncovered site (46%) compared to a covered dose site (~ 71%).

Disposition of EGEHE was generally consistent with previous studies investigating dermal exposure in other ethylene glycol ethers. A comparative dermal exposure study of EGME, EGEE, and EGBE in F344 rats demonstrated absorption (20–25%) and urinary excretion profiles similar to EGEHE (Sabourin et al., 1992). Another study comparing subcutaneous

and percutaneous of EGBE in Wistar rats found between 25–29% of EGBE was absorbed when applied topically to skin, with the majority of the absorbed dose recovered in urine within 24 h (Bartnik et al., 1987). Dermal exposure to EGHE, a close structural analog to EGEHE, has also been investigated. EGHE was rapidly absorbed with the majority of administered dose excreted via urine (33–45%), and feces (21–22%), and VOC (11–18%). Urinary excretion occurred primarily during the first 24 h after dermal application, which is consistent with that of EGEHE. Tissue distribution of EGHE was relatively low (<0.5%) for both species, and lower than what was observed in our study for rats (1.5–2%) following a 72-h covered dermal exposure (Ballantyne et al., 2003), but similar to what we observed in male and female mice.

For additional comparison, we investigated the clearance of EGEHE, EGBE, and EGHE in rat hepatocytes *in vitro*. Data from incubations in male and female hepatocytes indicated a faster intrinsic clearance for EGEHE, which is consistent with the extensive metabolism and rapid excretion observed in vivo. The rank order of clearance in hepatocytes was EGEHE > EGHE > EGBE. These findings indicate that hepatic clearance occurs more rapidly with increasing alkyl chain length in EGEs.

The urinary radiochromatograms demonstrated extensive metabolism of EGEHE in male and female rats and mice. Some differences were noted between dose routes and species although in general the pattern was similar across species, sexes and routes. A total of 14 peaks were detected, with little to no parent [^{14}C]EGEHE present in urine following gavage exposure. While most peaks represented a small fraction of the total peak area (≤5%), two peaks at 7.9 and 8.5 min did constitute more than 15% of the peak area. In contrast, fewer peaks (5 total) were detected following dermal application of 50 mg/kg in rats and mice, one of which corresponded to parent [^{14}C]EGEHE in male and female mice. The fewer observed peaks with dermal exposure could reflect differences between first-pass metabolism in the liver (and gastrointestinal tract) with oral exposure and cutaneous metabolism with topical application, possibly related to differential tissue expression of alcohol dehydrogenase (ADH) isoforms known to metabolize EGEs (Lockley et al., 2005, 2002).

Several analyses were conducted to characterize metabolites present in urine collected from male rats following gavage exposure to 500 mg/kg EGEHE. Enzymatic treatment with β-glucuronidase, sulfatase, and acylase resulted in modest shifts in larger peaks along with concomitant formation, shifts, or loss of less abundant peaks. The slight modifications to major peak areas observed in all three treatments, in the absence of a loss of a single major peak, suggest that these peaks likely represent multiple unresolved phase I or II modifications containing glucuronide, sulfate, or amino acid conjugates. An attempt to identify metabolites by LC-MS showed phase I metabolites prior to forming sulfate, mercapurate, or glucuronide conjugates. Glucuronide and sulfate metabolites have previously been observed in the urine of male F344 rats administered EGBE via gavage (Ghanayem et al., 1987b). Medinsky et al (1990) also observed glucuronidated but not sulfated metabolites in urine following drinking water exposure to EGBE. Glycine-conjugated metabolites have also been detected following exposure to EGEE via oral and dermal routes in male rats (Cheever et al., 1984; Lockley et al., 2002). Importantly, the conjugated metabolites referenced in these studies were present at significantly lower levels

than the alkoxyacetic acid metabolites. Given their known role in mediating the toxic effects of other EGEs, urine from EGEHE-treated male rats was screened for the presence of alkoxyacetic acid metabolites. Previous studies in EGME, EGEE, and EGBE have demonstrated that formation of alkoxyacetic acid metabolites increases with increasing alkyl chain length. Therefore, given the length of the EGEHE alkyl sidechain, we postulated that one or more alkoxyacetic acids would comprise a significant proportion of the EGEHE urinary metabolites. Interestingly, under the conditions used in our assessment, we did not identify a metabolite that matched standards for putative metabolites 2-EHOA, HOA, and MOA.

This highlights a potentially key difference between EGEHE and shorter EGEs, where alkoxyacetic acids formed via alcohol and aldehyde dehydrogenase (ALDH) oxidation pathway are the predominant metabolites. Glucuronidated metabolites have been identified in urine, although at much lower prevalence than the alkoxyacetic acid metabolite, following exposure to EGBE via dermal (Sabourin et al., 1992), oral gavage, and drinking water routes in male F344 rats (Ghanayem et al., 1987b; Medinsky et al., 1990). Further, co-exposure to EGBE and pyrazole, an ADH inhibitor, resulted in the increased formation of glucuronide metabolites rather than alkoxyacetic acid metabolites (Ghanayem et al., 1987a). Currently, no studies have characterized urinary metabolites for EGHE. A dermal exposure study in F344 rats demonstrated several urinary metabolites with little to no unmetabolized EGHE (Ballantyne et al., 2003); however, definitive metabolite identification was not conducted and thus it remains to be determined whether EGHE metabolism resembles or differs from that of EGEHE. However, the presence of glucuronidated metabolites with EGBE, and lack thereof following EGEE and EGME exposure, in conjunction with the observed sulfate and glucuronide conjugates in the present study may indicate a greater role of phase II conjugation pathways in addition to ADH/ALDH pathways with increasing alkyl chain length of EGEs.

Conclusion

To the authors' knowledge, the present study is the first to report disposition data for EGEHE in in the publicly available scientific literature. Our data demonstrate that EGEHE is well (oral gavage) or moderately (dermal application) absorbed in male and female rats and mice following a single administration and is excreted extensively via urine. EGEHE was distributed to tissues but the retention in tissues was low. EGEHE was extensively metabolized with numerous phase I and phase II modifications and very little, if any, parent EGEHE was detected in urine. There were no apparent dose, species or sex differences in excretion and metabolism of EGEHE, except greater amounts of EGEHE were detected in the exhaled VOC fraction in mice when compared to rats. These data will be used to inform subsequent toxicity studies aimed at evaluating the toxicological potential for EGEHE in rats and mice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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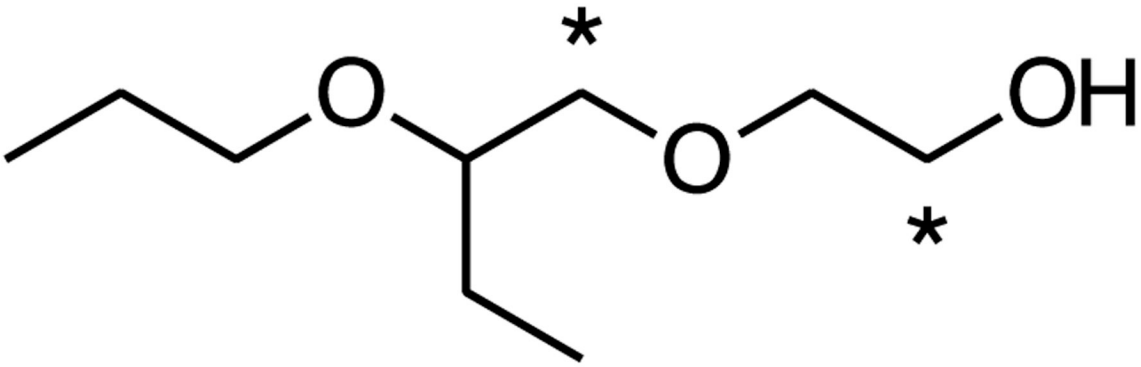

Figure 1.
Structure of [^{14}C]ethylene glycol 2-ethylhexyl ether (* Denotes the position of radioactivity)

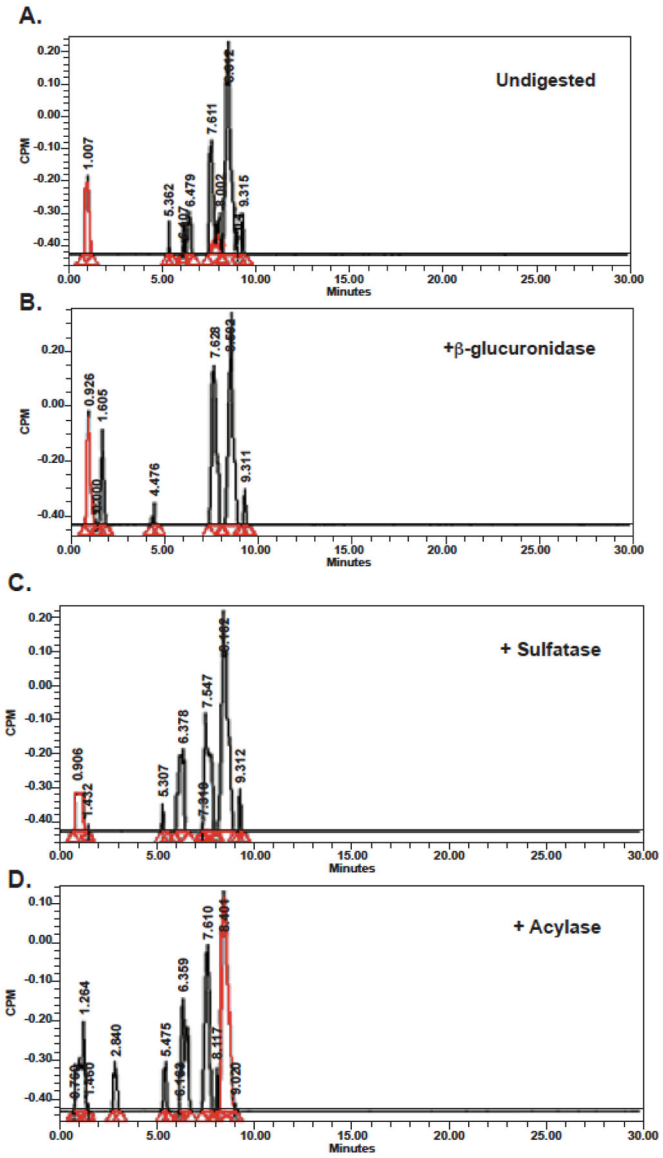

Figure 2.

HPLC analysis of urine from male Hsd:Sprague Dawley®SD® rats (0–72 h) following oral gavage of 500 mg/kg [^{14}C]ethylene glycol 2-ethylhexyl ether. Representative chromatograms are shown for urine: A) undigested, or incubated with B) β-glucuronidase, C) acylase, or D) sulfatase.

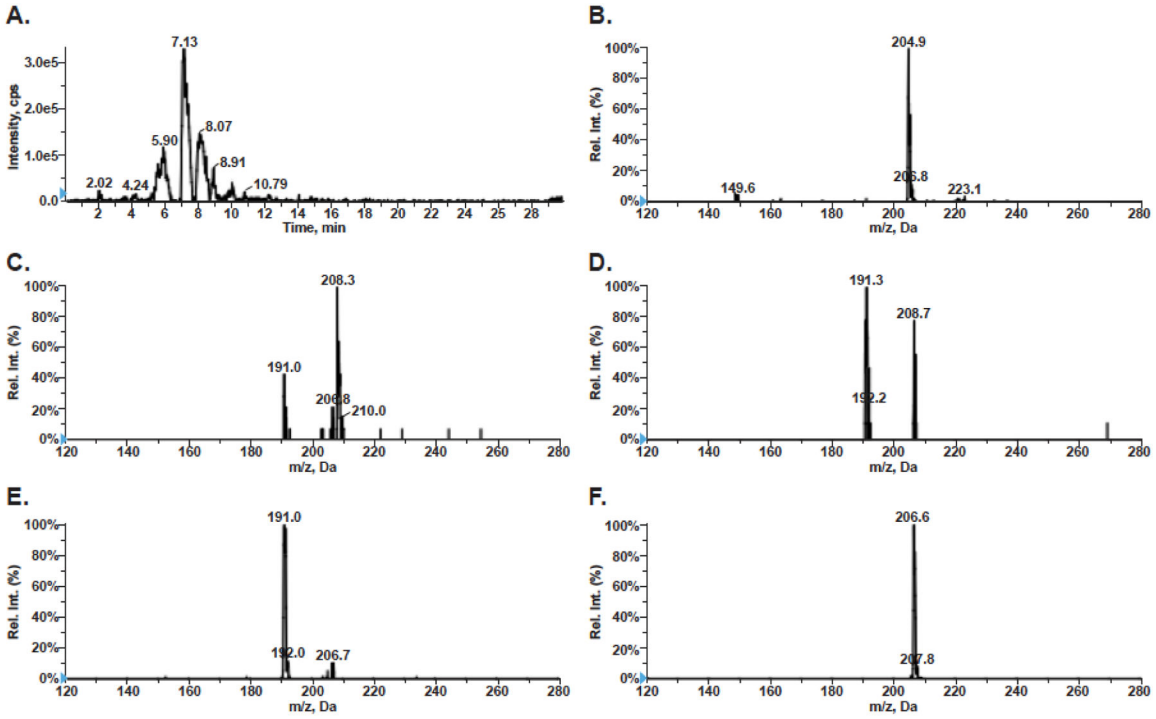

Figure 3.

LC-MS/MS positive neutral loss chromatograms of pooled urine (0–72h) from male rats administered 500 mg/kg [^{14}C]ethylene glycol 2-ethylhexyl ether via oral gavage. A) Chromatogram for loss of a sulfate ion (m/z 80) with 5 peaks shown. Mass spectra depicted for: B) Peak 1 eluting at ~ 6 min with m/z of 149.6 and 204.9, C) Peak 2 eluting at ~ 7 min with m/z of 191.0 and 208.3, D) Peak 3 eluting at ~ 8 min with ions m/z of 191.0 and 206.7, E) Peak 4 eluting at ~ 9 min with m/z of 191.0 and 206.7), and F) Peak 5 eluting at ~ 11 min with m/z of 206.6.

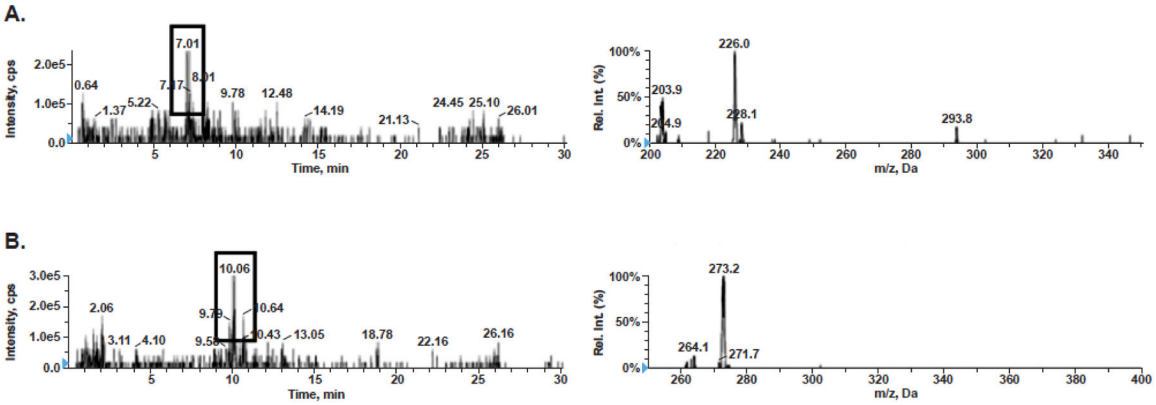

Figure 4.

LC-MS/MS negative ion mode neutral loss chromatograms of pooled urine (0–72h) from male rats administered 500 mg/kg. A) Extracted ion chromatogram for loss of a mercapurate moiety (m/z 129 m/z) and mass spectrum for a peak eluting at ~ 7 min with ions containing m/z of 226 and 294. B) Chromatogram for loss of a glucuronide moiety (m/z 176) and mass spectrum for a peak eluting at ~ 10 min with a m/z of 273.2.

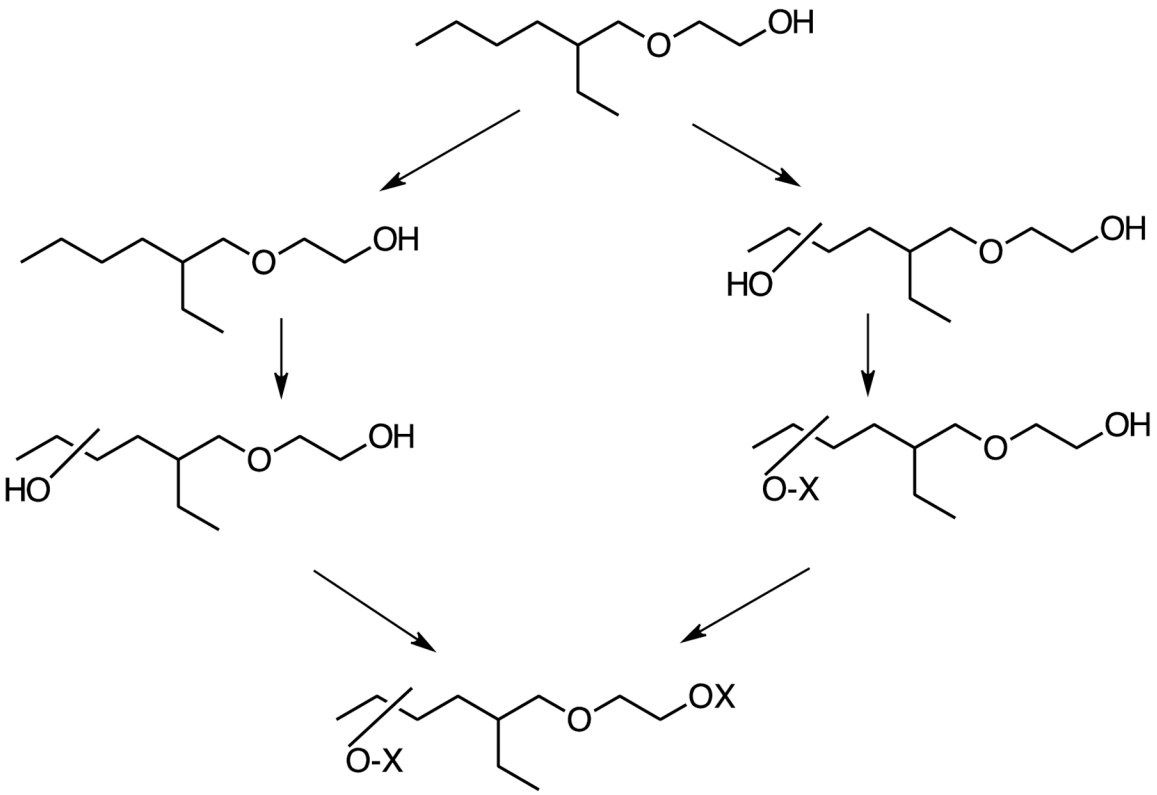

Figure 5.
Proposed general metabolic pathways of ethylene glycol 2-ethylhexyl ether in rodents. Data are based on analysis of rat urine. X=glucuronide, sulfate, or glycine.

Table 1.

Study design for [^{14}C]ethylene glycol 2-ethylhexyl ether in Hsd:Sprague Dawley®SD® rats and B6C3F1/N mice.

Species (Sex)	Dose (mg/kg)	Route	Study Duration (h)	Endpoint
Rat (M)	50, 150, 500	Gavage	48 or 72[a]	Dose response Mass balance Metabolism
Rat (F)	50	Gavage	48	Mass balance Sex differences
Rat (M)	50	Gavage	24	Tissue distribution
Rat (M)	50	Gavage	24	Biliary excretion
Rat (M)	50	Dermal (uncovered)	72	Mass balance
Rat (M, F)	50	Dermal (covered)	72	Mass balance
Mouse (M, F)	50	Gavage	72	Mass balance Species differences Sex differences
Mouse (M, F)	50	Dermal (covered)	72	Mass balance

[a]Male rats in the 50 and 150 mg/kg groups were sacrificed at 48h; 500 mg/kg male rats at 72 h.

Table 2.

Half-life of elimination and intrinsic clearance of EGEHE[a], EGHE, and EGBE in rat hepatocytes.

Parameter	EGEHE Male	EGEHE Female	EGHE Male	EGHE Female	EGBE Male
Elimination half-life (min)	4.23	4.39	45.5	17.6	79.3
Intrinsic clearance (mL/min/kg)	669	645	62.1	161	35.6

[a]Ethylene glycol 2-ethylhexyl ether, ethylene glycol hexylether, ethylene glycol butylether

Table 3.

Disposition of radioactivity following a single gavage administration of [^{14}C]ethylene glycol 2-ethylhexyl ether in male and female Hsd:Sprague Dawley®SD® rats.

Sample	Collection Interval (h)	24 h	48 h			72 h
		Male	Male	Female	Male	Male
		50 mg/kg	50 mg/kg	50 mg/kg	150 mg/kg	500 mg/kg
Urine	0 – 8	16.9 ± 1.7	17.6 ± 4.5	13.1 ± 8.5	20.5 ± 8.5	9.7 ± 6.6
	8 – 24	26.9 ± 8.8[a]	32.7 ± 7.4	30.5 ± 10.5	36.8 ± 4.9	22.8 ± 5.2
	24 – 48	-	7.0 ± 1.9[a]	1.7 ± 0.9[a]	4.4 ± 1.4[a]	3.8 ± 2.0
	48 – 72	-	-	-	-	0.4 ± 0.1[a]
Cage rinse	0 – 8	10.2 ± 5.1	6.5 ± 2.7	19.2 ± 11.4	7.3 ± 2.1	7.5 ± 2.6
	8 – 24	5.1 ± 2.6	3.1 ± 0.9	3.0 ± 0.3[b]	3.1 ± 1.3	8.7 ± 9.3
	24 – 48	-	1.5 ± 1.5	0.4 ± 0.2	0.5 ± 0.4	1.4 ± 1.4
	48 – 72	-	-	-	-	0.1 ± 0.01
Urine and cage rinsecage Cage	*Subtotal*	*59.1 ± 10.3*	*68.5 ± 8.4*	*67.9 ± 6.4*	*72.6 ± 5.6*	*54.4 ± 16.1*
Feces	0 – 24	12.8 ± 4.8	13.5 ± 6.3	8.6 ± 7.4	13.4 ± 7.6	12.1 ± 2.2
	24 – 48	-	3.2 ± 2.7	2.5 ± 0.5	0.9 ± 0.4	2.1 ± 1.2
	48 – 72	-	-	-	-	0.5 ± 0.03
	Subtotal	*12.8 ± 4.8*	*16.7 ± 8.4*	*11.1 ± 7.1*	*14.3 ± 7.5*	*14.3 ± 2.2*
GI contents[b]	-	7.1 ± 2.2	0.4 ± 0.1	0.2 ± 0.04	0.7 ± 0.3	0.2 ± 0.1
CO_2[c]	*Subtotal*	*2.8 ± 0.2*	*2.7 ± 0.5*	*3.2 ± 0.2*	*3.2 ± 0.2*	*4.2 ± 0.8*
Exhaled VOC[c]	*Subtotal*	*1.5 ± 2.7*	*0.5 ± 0.5*	*0.9 ± 1.1*	*0.4 ± 0.4*	*0.1 ± 0.03*
Tissues[b]		7.2 ± 2.0	4.6 ± 1.1	2.3 ± 0.4	4.8 ± 1.4	5.9 ± 2.0
Total recovered		90.4 ± 4.8	93.3 ± 3.5	85.5 ± 1.6	96.0 ±9.3	79.5 ± 17.0

Data shown are mean (± SD) percent radioactivity recovered.

N = 4 for all dose groups, except 500 mg/kg (N = 3).

[a]Includes urine present in bladder at time of sacrifice.

[b]Collected at study termination, which was either 24, 48, or 72 h. GI, gastrointestinal.

[c]Subtotals are given for routes with low excretion.

Table 4.

Tissue concentration (ng-equivalents/g tissue) following a single gavage administration of [^{14}C]ethylene glycol 2-ethylhexyl ether to male and female Hsd:Sprague Dawley®SD® rats.

Tissue[a]	Male 50 mg/kg 24 h		Male 50 mg/kg 48 h		Female 50 mg/kg 48 h		Male 150 mg/kg 48 h		Male 500 mg/kg 72 h	
	Conc	TBR[b]	Conc.	TBR	Conc.	TBR	Conc.	TBR	Conc.	TBR
Blood	168	-	5000	-	10.8	-	21,556	-	40,400	-
Plasma	-	-	2450	0.5	-	-	-	-	-	-
Adipose	296	1.9	1700	0.3	4.8	0.5	3530	0.2	14,300	0.4
Muscle	101	0.6	1630	0.4	3.8	0.4	8840	0.7	23,200	0.6
Skin	125	0.8	1560	0.4	6.3	0.7	4440	0.3	51,000	1.3
Spleen	189	1.2	6010	1.2	18.8	2.1	35,000	2.5	68,000	1.7
Liver	896	5.4	15,000	3.2	40.1	4.5	33,400	2.1	145,000	3.7
Kidney	369	2.3	4750	1.1	19.2	2.1	16,900	1.0	67,700	1.7
Bladder	3250	21.9	5910	1.4	14.1	1.4	29,600	1.6	73,400	1.9
Brain	87.2	0.5	1310	0.3	3.1	0.3	3980	0.2	11,800	0.3
Heart	188	1.1	3450	0.8	10.4	1.1	31,400	2.2	44,200	1.1
Lung	475	2.4	4030	0.9	14.5	1.5	19,500	1.1	59,500	1.5
Stomach	532	3.1	4090	0.9	15.4	1.6	14,700	0.9	53,900	1.4
Large intestine	1300[c]	8.2	5080[c]	1.1	17.4	1.7	23,900[c]	1.4	62,200	1.6
Small intestine					22.2	2.4			68,200	1.7
Thyroid	378	2.3	5320	1.3	25.2	2.7	22,100	1.1	114,000	2.9
Pancreas	574	3.4	4130	0.9	19.0	2.1	21,800	1.4	84,200	2.1
Testes/ovaries	221	1.2	1800	0.4	30.0	3.5	4100	0.3	19,000	0.5

Data shown are mean (± SD) N = 4/treatment group, except 500 mg/kg (N = 3).

[a] Tissue weights were calculated using the following percentages of body weight: adipose 7.0%, blood 7.4%, muscle 40.4%, and skin 19% (ILSI, 1994); Brown et al., 1997).

[b] TBR = tissue-to-blood ratio. TBR for each individual animal was calculated using corresponding tissue and blood data and the average for the group is shown here.

[c] Large and small intestine were measured together.

Table 5.

Disposition of total radioactivity recovered at 72 h following a single dermal application of 50 mg/kg [^{14}C]ethylene glycol 2-ethylhexyl ether in male and female Hsd:Sprague Dawley®SD® rats and B6C3F1/N mice.

Sample	Collection Interval (h)	Hsd:Sprague Dawley®SD® rats			B6C3F1 mice	
		Male (uncovered)	Male (covered)	Female (covered)	Male (covered)	Female (covered)
Urine[a]	4 – 8	6.55 ± 1.78	8.46 ± 2.81	8.22 ± 4.32	0.23 ± 0.43	3.51 ± 6.04
	12 – 24	6.22 ± 1.46	12.58 ± 1.59	7.93 ± 2.00	1.80 ± 2.24	1.56 ± 1.35
	24 – 48	1.60 ± 0.18	2.40 ± 0.98	1.20 ± 0.42	0.37 ± 0.11	0.34 ± 0.23
	48 – 72	0.31 ± 0.11	0.43 ± 0.21	0.40 ± 0.18	0.19 ± 0.08	0.12 ± 0.10
Cage Rinse	4 – 8	2.54 ± 0.50	5.03 ± 1.77	8.81 ± 3.14	7.28 ± 4.15	4.32 ± 2.06
	12 – 24	1.44 ± 0.85	1.61 ± 0.72	1.29 ± 0.29	3.06 ± 1.33	1.51 ± 0.69
	24 – 48	0.54 ± 0.20	0.39 ± 0.21	0.44 ± 0.18	0.74 ± 0.27	0.62 ± 0.48
	48 – 72	0.06 ± 0.02	0.19 ± 0.10	0.30 ± 0.13	0.55 ± 0.43	0.58 ± 0.56
Urine + cage rinse	*Subtotal*	*19.30 ± 1.59*	*31.10 ± 4.63*	*28.6 ± 2.00*	*14.2 ± 4.34*	*12.5 ± 4.06*
Feces	0 – 24	2.66 ± 0.57	1.55 ± 0.85	1.88 ± 0.34	2.77 ± 2.20	6.10 ± 2.73
	24 – 48	0.89 ± 0.39	0.94 ± 0.68	1.08 ± 0.83	0.60 ± 0.44	1.00 ± 0.43
	48 – 72	0.19 ± 0.06	0.44 ± 0.23	0.37 ± 0.14	1.04 ± 0.72	1.10 ± 1.16
	Subtotal	*3.74 ± 0.72*	*2.93 ± 1.24*	*3.34 ± 0.71*	*4.42 ± 3.11*	*8.20 ± 2.14*
VOC	0 – 4	3.33 ± 0.23	4.10 ± 2.07	5.81 ± 1.74	6.69 ± 1.14	10.07 ± 2.00
	4 – 8	3.86 ± 0.83	4.57 ± 0.74	6.61 ± 1.89	7.44 ± 0.47	6.79 ± 1.24
	8 – 12	2.55 ± 0.52	3.33 ± 0.60	4.14 ± 0.78	5.70 ± 0.52	6.02 ± 0.86
	12 – 24	3.81 ± 0.32	6.22 ± 1.54	8.80 ± 0.75	11.59 ± 1.78	10.45 ± 0.89
	24 – 48	4.12 ± 0.90	8.00 ± 1.73	8.55 ± 0.50	11.06 ± 4.07	13.67 ± 1.02
	48 – 72	3.18 ± 0.78	5.89 ± 0.70	5.12 ± 0.76	11.05 ± 2.10	11.19 ± 0.85
	Subtotal	*20.84 ± 1.94*	*32.10 ± 4.72*	*39.03 ± 4.42*	*53.60 ± 5.95*	*58.20 ± 5.69*
CO_2	*Subtotal*	*0.85 ± 0.13*	*1.68 ± 0.49*	*1.49 ± 0.29*	*2.21 ± 0.93*	*1.95 ± 1.02*
GI contents[b]	-	0.17 ± 0.08	0.10 ± 0.02	0.19 ± 0.18	0.08 ± 0.04	0.11 ± 0.07
Tissues	-	0.91 ± 0.16	1.57 ± 0.47	2.16 ± 1.99	0.69 ± 0.20	0.74 ± 0.18
Dose site swipes	-	0.04 ± 0.02	1.93 ± 0.44	4.01 ± 2.24	8.18 ± 1.91	2.69 ± 0.52
Dose site skin	-	0.5 ± 0.2	0.03 ± 0.01	0.46 ± 0.18	2.19 ± 1.26	0.55± 0.08
Total unabsorbed dose	-	*21.30 ± 1.94*	*34.10 4.80*	*43.50 ± 5.87*	*62.2 ± 6.62*	*61.4 ± 5.69*
Total absorbed dose[c]	-	*24.90 ± 1.83*	*37.40 ± 5.81*	*35.80 ± 1.41*	*21.6 ± 1.99*	*23.5 ± 2.6*
Total recovered dose	-	*46.30 ± 3.35*	*71.40 ± 4.77*	*79.3 ± 7.28*	*83.9 ± 7.35*	*84.9 ± 6.52*

Data shown are mean (± SD) percent radioactivity recovered (N = 4/group).

[a]Includes urine present in bladder at time of sacrifice.

[b]Collected at study termination (72 h). GI, gastrointestinal.

[c] Absorbed dose includes percent recovered in feces, urine, and tissues. VOC were not included due to potential evaporation of [^{14}C]EGEHE from the dose site.

Table 6.

Tissue concentration (ng-equivalents/g tissue) at 72 h following a single dermal application of 50 mg/kg [^{14}C]ethylene glycol 2-ethylhexyl ether to male and female Hsd:Sprague Dawley®SD® rats

Tissue[a]	Male (uncovered) 50 mg/kg		Male (covered) 50 mg/kg		Female (covered) 50 mg/kg	
	Conc.	TBR[b]	Conc.	TBR	Conc.	TBR
Blood	2.88	-	6.92	-	8.24	-
Adipose	2.78	1.0	3.92	0.6	3.56	1.1
Muscle	2.70	0.9	3.45	0.5	5.04	1.8
Skin	2.88	1.0	3.23	0.5	6.94	2.6
Spleen	5.92	1.4	10.14	1.6	9.99	1.1
Liver	11.75	4.1	23.04	3.7	26.54	3.5
Kidney	6.31	2.2	9.09	1.4	6.31	1.8
Bladder	11.91	4.1	15.06	2.0	7.85	2.5
Brain	1.59	0.6	4.14	0.6	3.54	1.1
Heart	4.24	1.5	6.04	0.9	3.57	1.0
Lung	6.23	2.2	11.77	1.9	14.83	5.1
Stomach	5.66	2.0	6.63	1.0	5.29	1.5
Small and large intestines	7.68	2.7	10.64	1.6	6.37	2.0
Thyroid	11.58	4.0	11.71	1.7	20.18	7.5
Pancreas	6.29	2.2	6.91	1.0	5.14	1.5
Testes/ovaries	2.22	0.8	3.97	0.6	5.56	1.6

Data shown are mean (N = 4/group).

[a]Tissue weights were calculated using the following percentages of body weight: adipose 7.0%, blood 7.4%, muscle 40.4%, and skin 19% (Brown et al., 1997)(ILSI, 1994).

[b]TBR = Tissue to blood ratio.

Table 7.

Disposition of radioactivity 48 h following gavage administration of 50 mg/kg [^{14}C]ethylene glycol 2-ethylhexyl ether to male and female B6C3F1/N mice.

Sample	Collection Interval (h)	Percent of dose	
		Male	Female
Urine	4 – 8	<0.01 ± <0.01	3.0 ± 3.8
	12 – 24	2.8 ± 2.9	10.0 ± 19.4
	24 – 48[a]	1.6 ± 1.2	0.5 ± 0.4
Cage rinse	4 – 8	5.3 ± 4.4	21.3 ± 16.3
	12 – 24	11.1 ± 4.2	10.0 ± 8.2
	24 – 48	6.2 ± 2.7	3.7 ± 1.2
Urine + cage rinse	*Subtotal*	*27.0 ± 1.1*	*47.6 ± 13.9*
Feces	0 – 24	28.8 ± 17.5	14.6 ± 8.0
	24 – 48	7.8 ± 3.4	2.6 ± 2.5
	Subtotal	*36.5 ± 14.9*	*17.2 ± 10.0*
GI tract contents[b]	-	0.1 ± 0.01	0.2 ± 0.1
CO_2	0 – 4	1.5 ± 0.9	2.7 ± 0.7
	4 – 8	0.5 ± 0.2	1.7 ± 0.5
	8 – 12	0.9 ± 0.3	0.6 ± 0.2
	12 – 24	0.7 ± 0.1	0.7 ± 0.4
	24 – 48	0.7 ± 0.2	0.3 ± 0.1
	Subtotal	*4.2 ± 0.4*	*5.8 ± 0.7*
Exhaled VOC	0 – 4	1.8 ± 0.4	2.0 ± 0.1
	4 – 8	2.0 ± 1.2	1.9 ± 1.0
	8 – 12	4.6 ± 3.0	2.7 ± 2.5
	12 – 24	5.0 ± 3.0	5.2 ± 3.3
	24 – 48	7.9 ± 5.0	8.3 ± 5.2
	Subtotal	*18.7 ± 2.6*	*20.1 ± 12.0*
Tissues[b]		4.1 ± 0.8	3.8 ± 0.9
Total recovered		**90.5 ± 19.3**	**94.7 ± 7.1**

Data are mean ± SD percent radioactivity recovered (N = 4 / group).

[a]Includes urine present in bladder at time of sacrifice.

[b]Collected at study termination (48 h). GI, gastrointestinal.

Table 8.

Tissue concentrations (ng-equivalents/g tissue) following gavage administration (48 h) and dermal application (72 h) of 50 mg/kg [[^{14}C]ethylene glycol 2-ethylhexyl ether to male and female B6C3F1/N mice.

	Oral, 50 mg/kg [^{14}C]EGEHE				Dermal (covered), 50 mg/kg [^{14}C]EGEHE			
	Male		Female		Male		Female	
Tissue[a]	Conc.	TBR[b]	Conc.	TBR	Conc.	TBR	Conc.	TBR
Blood	11.4	-	9.9	-	2.26	-	1.73	-
Adipose	6.2	0.5	11.0	1.2	2.05	0.9	1.96	1.
Muscle	8.4	0.8	8.3	0.9	1.73	0.8	1.90	1.1
Skin	12.4	1.2	12.3	1.3	2.57	1.1	2.54	1.5
Spleen	26.8	5.8	23.9	2.3	6.8	2.9	5.77	3.4
Liver	87.6	7.9	63.5	6.5	10.81	4.7	8.85	5.1
Kidney	38.3	3.5	28.2	2.8	10.96	4.8	5.86	3.4
Bladder	53.4	4.7	31.7	3.1	9.58	4.1	10.76	6.5
Brain	5.0	0.5	5.4	0.6	1.32	0.6	1.23	0.7
Heart	19.4	1.8	41.4	4.7	5.56	2.4	4.19	2.5
Lung	30.1	2.8	22.5	2.3	7.09	3.0	6.87	4.1
Stomach	35.9	3.3	ND[b]	ND	6.43	2.8	5.08	3.0
Small and large intestines	42.2	4.1	133	3.2	8.94	3.7	4.84	2.9
Thyroid	36.3	3.3	28.5	2.9	9.34	4.1	6.61	4.0
Pancreas	32.4	3.0	23.9	2.4	5.76	2.5	4.64	2.8
Testes/ovaries	12.3	1.1	36.9	3.5	2.11	0.9	6.72	4.3

Data shown are mean (N= 4/treatment group).

[a]Tissue weights were calculated using the following percentages of body weight: adipose 7.0%, blood 7.4%, muscle 40.4%, and skin 19% (ILSI, 1994); Brown et al., 1997).

[b]TBR = Tissue-to-blood ratio. TBR for each individual animal was calculated using corresponding tissue and blood data and the average for the group is shown here; ND = not determined