DISPOSITION OF 1,2-[\textsuperscript{14}C]DIBROMOETHANE IN MALE WISTAR RATS

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ABSTRACT:

In this study the disposition of 1,2-[\textsuperscript{14}C]dibromoethane (1,2-[\textsuperscript{14}C]DBE) was investigated in male Wistar rats. 1,2-DBE is a cytotoxic and carcinogenic compound that has been used as an additive in leaded gasoline and as a soil fumigant. 1,2-[\textsuperscript{14}C]DBE was administered orally or iv. Radioactivity was recovered (mostly within 48 hr after administration) in urine (75–82% of the dose), feces (3.2–4% of the dose), and expired air (0.53–7.2% of the dose). One hundred–sixty-eight hours after administration of 1,2-[\textsuperscript{14}C]DBE, most of the radioactivity in tissues was found in the liver, lungs, and kidneys (<1% of the dose) and the red blood cells (0.3% of the dose). Identified urinary metabolites were S-(2-hydroxyethyl)mercapturic acid, thiadiacetic acid, and thiadiacetic acid sulfoxide, together accounting for, on average, 78% of the total amount of radioactivity in urine. In addition to S-(2-hydroxyethyl)mercapturic acid, thiadiacetic acid, and thiadiacetic acid sulfoxide, several compounds were anticipated as potential urinary metabolites of 1,2-DBE, i.e. S-(2-hydroxyethyl)thiolactic acid, S-(2-hydroxyethyl)thiopurinic acid, S-(2-hydroxyethyl)thiolactic acid, and S-(2-hydroxyethyl)thiolactic acid. All of the postulated urinary metabolites were synthesized and searched for in urine samples. None of these metabolites could be detected in urine, however. The data obtained in the present study might be useful for risk assessment and biomonitoring studies of 1,2-DBE and will also be used to further validate a physiologically based pharmacokinetic model for 1,2-DBE in rats and humans that was recently developed.

1,2-DBE\textsuperscript{1} is used as an additive in leaded gasoline and as a soil fumigant (for review, see Alexeeff et al., 1990). In addition to causing toxicity in a variety of organs (Alexeeff et al., 1990), 1,2-DBE has been shown to be genotoxic in a variety of test systems (Rannug, 1980; van Bladeren et al., 1980; Kerklaan et al., 1985; Zoetemek et al., 1987; Abril et al., 1995; Graves et al., 1996). Furthermore, tumor formation has been observed in a number of studies with experimental animals, both at the site of application and at distant sites. Tumors and proliferative lesions have been found in the stomach after oral administration (Olson et al., 1973; Powers et al., 1975; Weisburger, 1977), in the nasal cavities and lungs after inhalation (Stinson et al., 1981; Huff, 1983; Wong et al., 1982), and in the skin after dermal application (van Duuren et al., 1979). Based on animal studies, 1,2-DBE is suspected to be a carcinogen in humans, although unequivocal evidence is still lacking (Ramsey et al., 1979; Ott et al., 1980; Apfeldorf and Infante, 1981; Sweeney et al., 1986).

The metabolism of 1,2-DBE is mediated by both P450 and GST enzymes. The major P450 isoenzyme metabolizing 1,2-DBE in rats and humans has been identified as CYP2E1 (Guengerich et al., 1991; Wormhoudt et al., 1996). Two other P450 isoenzymes (CYP2A6 and CYP2B6) were also found to oxidize 1,2-DBE, but with much lower catalytic efficiency (Wormhoudt et al., 1996). Upon oxidation of 1,2-DBE by P450, 2-BA is formed; it may bind to cellular proteins or, alternatively, be conjugated to GSH and enter the mercapturic acid pathway (Hill et al., 1978; van Bladeren et al., 1981; Vermeulen, 1989; Commandeur et al., 1995).

To date, in both rats and humans, four classes of GSTs have been shown to conjugate 1,2-DBE to GSH. The α-class GSTs were found to have higher activity toward 1,2-DBE, compared with the μ- and π-class isoenzymes (Stokoe et al., 1990; Ploem et al., 1997). Recently, the rat GST5-5 and human GSTT1-1 θ-class isoenzymes were shown to possess far higher activity toward 1,2-DBE, compared with the rat and human α-, μ-, and π-class isoenzymes, suggesting that the θ-class GSTs may play the most important role (Ploem et al., 1997). Upon GST-catalyzed metabolism, a reactive episulfonium ion is formed, which is able to react with the N\textsuperscript{2}-position in guanine DNA bases and is suggested to be involved in the genotoxic effects of 1,2-DBE (van Bladeren et al., 1980; Peterson et al., 1988). Several in vitro studies have shown that the products generated from 1,2-DBE by GST enzymes bind preferentially to DNA, whereas the products from the P450 pathway bind preferably to proteins (Shih and Hill, 1981; Hissink AM and Sherratt P, personal communication.

\textsuperscript{1} Abbreviations used are: 1,2-DBE, 1,2-dibromoethane; 2-BA, 2-bromoacetaldelyde; CMC, S-(carboxymethyl)-L-cysteine; CM-LACT, S-(carboxymethyl)-thiolactic acid; CM-MA, S-(carboxymethyl)mercapturic acid; CM-PYR, S-(carboxymethyl)thiopurinic acid; 2-HEMA, S-(2-hydroxyethyl)-mercapturic acid; HE-LACT, S-(2-hydroxyethyl)-thiolactic acid; 2-HEMA, S-(2-hydroxyethyl)-mercapturic acid; HE-PYR, S-(2-hydroxyethyl)thiopurinic acid; HE-TA, S-(2-hydroxyethyl)thioacetic acid; PBPK model, physiologically based pharmacokinetic model; P450 or CYP, cytochrome P450; TDA, thiadiacetic acid; TDA-SO, thiadiacetic acid sulfoxide; TFA, trifluoroacetic acid.

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Sundheimer et al., 1982), thus supporting the view that the GST-mediated bioactivation of 1,2-DBE is involved in its genotoxicity (van Bladeren et al., 1980). However, some binding to DNA has also been observed in vitro upon bioactivation of 1,2-DBE by P450 enzymes (Wiersma et al., 1986), and the product of this bioactivation, 2-BA, has been reported to bind to DNA in vitro (Banerjee et al., 1979). Therefore, a role for P450-mediated bioactivation of 1,2-DBE in its genotoxic effects cannot be ruled out.

In our laboratories, 1,2-DBE has been used as a model compound in studies on interindividual variability in metabolism and in studies in which in vitro metabolic parameters were used to construct a PBPK model to aid in human risk assessment for 1,2-DBE (Wormhoudt et al., 1996; Ploemen et al., 1995, 1997). Large interindividual variability was observed in both P450- and GST-mediated metabolism of 1,2-DBE in human liver samples (Wormhoudt et al., 1996; Ploemen et al., 1997). Furthermore, the GST-mediated bioactivation of 1,2-DBE was found to be influenced by the GSTT1–1 \( \theta \)-class polymorphism in human erythrocytes, indeed indicating the important role of \( \theta \)-class GSTs in the bioactivation process (Ploemen et al., 1995).

The aim of the present study was to comprehensively investigate the in vivo disposition of 1,2-DBE in rats. For this purpose, 1,2-[\(^{14}\)C]DBE was administered to rats by two routes of administration (i.e. orally and iv) and its disposition was studied in urine, feces, expired air, and tissues. The data obtained from this study might be useful for risk assessment and biomonitoring studies of 1,2-DBE and will be used to further validate a PBPK model that was recently developed for 1,2-DBE in rats and humans (Ploemen et al., 1997). This PBPK model includes interindividual variability in biotransformation in human risk assessment for this carcinogenic compound.

### Animals, Materials, and Methods

**Animals.** The animals used in this study were adult male Wistar rats [strain Crl:WI(WU)BR] obtained from Charles River Wiga (Sulzfeld, Germany). At the time the experiments were carried out, the rats had body weights of 270–300 g. The animals had free access to tap water and a cereal-based rodent diet (SDS Special Diets Services, Witham, England) throughout the experiment.

**Chemicals.** 1,2-[\(^{14}\)C]DBE (specific activity, 17.9 mCi/mmol; radiochemical purity, >99%), cysteine, amino acid oxidase, and catalase were obtained from Sigma Chemical Co. (St. Louis, MO). Unlabeled 1,2-DBE, 2-mercaptoethanol, 2-mercaptoacetic acid, CMC, and NaBH\(_4\) were obtained from Aldrich (Milwaukee, WI). Intralipid (30%) was obtained from Pharmacia (Uppsala, Sweden). TDA, 2-bromoacetic acid, 2-iodoacetic acid, and 2-bromoethanol were obtained from Merck (Darmstadt, Germany). Bromopyruvic acid was obtained from Vetrion Alpha Products (Karlsruhe, Germany). 2-HEMA was synthesized on an analytical scale to screen for their presence in urine. All anticipated products were analyzed by GC/MS. Before GC/MS analysis, samples were treated with ethereal diazomethane to methylate the carboxyl groups of the compounds. To confirm the chemical identities of the novel reference compounds, they were synthesized by two independent synthetic routes.

**HE-PYR.** HE-PYR was synthesized both chemically and enzymatically. In method A (synthetic method), 200 \( \mu \)mol of mercaptoethanol and 470 mg of bromopyruvic acid were dissolved in 5 ml of dimethylsulfoxide. The reaction was started by addition of 500 \( \mu \)l of triethylamine. After 3 hr at room temperature, a 1-ml sample was added to 10 ml of 2 N hydrochloric acid and extracted with 10 ml of ethyl acetate. The ethyl acetate was evaporated, and the residue was treated with diazomethane. The GC/MS spectrum of the major product was consistent with the dimethylated form of HE-PYR (because of keto-enolization of the thioypyrinic acid moiety) (Commandeur et al., 1996). In method B (enzymatic method), 4 mmol 2-HEC in 2 ml of 50 mm potassium phosphate buffer (pH 7.4) was incubated at 37°C in the presence of 1 mg/ml amino acid oxidase and 40 units/ml catalase. After 2 hr, the incubation mixture was acidified to pH 2 and extracted with 3 ml of ethyl acetate. The ethyl acetate fraction was evaporated to dryness and treated with diazomethane. GC/MS analysis demonstrated a major peak with a retention time and mass spectrum identical to those for the product formed using method A [GC/MS data: retention time, 9.06 min; \( m/z \) 192 (\( M^+ \), 0.4%), 174 (\( M^+ -\text{H}_2\text{O} \), 5.9%), 147 (\( M^+ -\text{CH}_2\text{OH} \), 10%), 131 (14%), and 115 (\( M^+ -\text{SCH}_2\text{CH}_2\text{OH} \), 100%)].

**CM-PYR.** CM-PYR was synthesized by procedures identical to those used for HE-PYR, as described above. In the chemical procedure (method A), 2-mercaptoacetic acid was used instead of 2-mercaptoethanol. In the enzymatic procedure (method B), CMC was used instead of 2-HEC. The two methods resulted in formation of identical products, according to the identical retention times and mass spectra from GC/MS analysis of methylated extracts. The mass spectrum was consistent with the dimethylated form of CM-PYR [GC/MS data: retention time, 9.52 min; \( m/z \) 220 (\( M^+ \), 0.7%), 161 (\( M^+ -\text{COOH} \), 1.8%), 147 (\( M^+ -\text{CH}_2\text{COOCH}_3 \), 3%), and 115 (\( M^+ -\text{SCH}_2\text{COOH} \), 21%)].

**HE-TA.** HE-TA was synthesized by two different synthetic methods. In method A, 2.5 mmol of 2-mercaptoacetic acid and 2.5 mmol of 2-bromoethanol were dissolved in 5 ml of dimethylsulfoxide. The reaction was started by addition of 500 \( \mu \)l of triethylamine. After 3 hr at room temperature, a 1-ml sample was added to 10 ml of 2 N hydrochloric acid and extracted with 10 ml of ethyl acetate. The ethyl acetate fraction was evaporated to dryness and treated with diazomethane. GC/MS analysis demonstrated a major peak with a retention time and mass spectrum identical to those for the product formed using method A [GC/MS data: retention time, 9.06 min; \( m/z \) 192 (\( M^+ \), 0.4%), 174 (\( M^+ -\text{H}_2\text{O} \), 5.9%), 147 (\( M^+ -\text{CH}_2\text{OH} \), 10%), 131 (14%), and 115 (\( M^+ -\text{SCH}_2\text{CH}_2\text{OH} \), 100%)].

**Synthesis and Characterization of Anticipated GSH-Derived Metabolites of 1,2-DBE.** In our laboratories, 1,2-DBE has been used as a model compound in studies on interindividual variability in metabolism and in studies in which in vitro metabolic parameters were used to construct a PBPK model to aid in human risk assessment for 1,2-DBE by P450 enzymes (Wiersma et al., 1986), and the product of this bioactivation, 2-BA, has been reported to bind to DNA in vitro (Banerjee et al., 1979). Therefore, a role for P450-mediated bioactivation of 1,2-DBE in its genotoxic effects cannot be ruled out.

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 acetate was evaporated, and the residue was treated with diazomethane. GC/MS analysis of products obtained by the two methods revealed one major peak, with a mass spectrum consistent with HE-TA methyl ester. A minor peak was identified as S-(2-methoxyethyl)thioacetic acid methyl ester, resulting from the relatively slow methylation of the 2-hydroxy group [GC/MS data for HE-TA methyl ester: retention time, 7.04 min; m/z 150 (M+, 0.25%), 132 (M+-H2O, 37%), 120 (7%), 104 (M+-CH3CH2OH, 29%), 91 (M+-COOCH3, 49%), and 74 (100%); GC/MS data for S-(2-methoxyethyl)thioacetic acid methyl ester: retention time, 6.87 min; m/z 164 (M+, 2%), 132 (100%), 119 (M+-CH3OCH2, 6%), 105 (M+-COOCH3 and/or M+-CH2CH2OCH3, 27%), and 91 (M+-CH2COOCH3, 25%)].

HE-LACT. HE-LACT was synthesized by reduction of chemically synthesized HE-PYR with NaBH4 (method A) and by diazotation hydrolysis of 2-HEC (method B). In method A, 200 µl of a solution of 100 mg/ml NaBH4 was added to 1 ml of a solution methyl ester: retention time, 6.87 min; m/z 164 (M+, 2%), 132 (100%), 119 (M+-CH3OCH2, 6%), 105 (M+-COOCH3 and/or M+-CH2CH2OCH3, 27%), and 91 (M+-CH2COOCH3, 25%).

The thiolactate moiety) [GC/MS data: retention time, 9.13 min; m/z 170 (M+, 100%), 132 (M+-H2O, 3%), 162 (M+-HCl, 5%), 180 (M+-COOCH3 and/or M+-CH2CONH2, 4%), 144 (M+-CICH2CH2S, 65%), 138 (13%), 117 (M+-CICH2SCH2OH and/or COOCH3) or M+-CICH2CH2 and CH2CONH2), 19%, 113 (14%), and 88 (100%); GC/MS data for S-(2-chloroethyl)thiolactic acid methyl ester (from HE-LACT and expected from its sulfoxide): retention time, 8.72 min; m/z 180 (M+-H2O, 3%), 162 (M+-HCl, 7%), 144 (M+-HCOONH4, 18%), 139/141 (M+-COOCH3, 12/3.6%), and 109/111 (M+-CHOHCOOCH3, 100/48%); GC/MS data for S-(2-chloroethyl)thiolactic acid methyl ester (from HE-LACT and expected from its sulfoxide): retention time, 6.99 min; m/z 132 (M+-HCl, 28%), 109/111 (M+-COOCH3, 25/8%), and 74 (100%).

Animal Experiments. Rats were housed in metabolism cages throughout the experiment, which was carried out in an air-conditioned room with a light/dark cycle of 12 hr. Four groups of rats (N = 4 for each group) were used; animals in group A were treated orally with 50 mg/kg 1,2-[14C]DBE dissolved in safflower oil, those in group B were treated orally with 150 mg/kg 1,2-[14C]DBE dissolved in safflower oil, those in group C were treated iv with 10 mg/kg 1,2-[14C]DBE dissolved in Intralipid, and those in group D were treated iv with 50 mg/kg 1,2-[14C]DBE dissolved in Intralipid. The specific activities of solutions of 1,2-[14C]DBE mixed with unlabeled 1,2-DBE, as administered to the animals, were 1.71 · 108 dpm/mg, 9.23 · 109 dpm/mg, 5.72 · 109 dpm/mg, and 1.66 · 109 dpm/mg for groups A, B, C, and D, respectively. The amount of radioactivity was determined immediately in the expired air at 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hr after administration of 1,2-[14C]DBE, by forcing the expired air to flow through two bottles, of which one was filled with dimethylsulfoxide and the other with ethanol. Pumps were used to force air to flow through the metabolism cages and the bottles with organic solvents. Most of the radioactivity was found in the first bottle, which contained dimethylsulfoxide. Feces and urine were collected at 4, 8, 24, 48, 72, 96, 120, 144, and 168 hr after administration of 1,2-[14C]DBE. Urine was collected on dry ice for the first 24 hr. The feces were homogenized in water in a volume equaling approximately 3 times the weight of the feces. After 168 hr, the animals were killed by aortal bleeding under ether anesthesia, after which the liver, kidneys, stomach, lungs, perirenal fat, skin, small intestine, bladder, and spleen were removed from the animals. The stomach and small intestine were rinsed thoroughly. Blood was centrifuged and separated into plasma
and red blood cells. The residual carcass was dissolved in 1.5 M KOH in 20% (v/v) ethanol by heating for 24 hr at 60°C. The metabolism cages were washed with approximately 70 ml of 0.5% (v/v) Triton X-100.

**Measurement of Radioactivity.** Samples of urine (0.5 ml), plasma (0.5 ml), and cage washes (0.7 ml) were mixed with 4.5 ml of Ultima Gold scintillation cocktail (Packard Instrument Co., Reading, UK), residual carcass (1 ml) was mixed with 15 ml of Hionic Fluor scintillation cocktail (Packard), and samples (1 ml) of the solvents containing radioactivity from the expired air (see above) were mixed with 15 ml of Ultima Gold scintillation cocktail. Radioactivity was measured in a liquid scintillation analyzer (Packard Instrument Co.), using automatic external standard quench correction. Samples (approximately 0.5 g) of feces, blood, and tissue were weighed in combustible cups and burned in oxygen using an automatic sample oxidizer (model 307 Mk2; Packard Instrument Co.). The combustion products were absorbed in CarboSorb absorbant and mixed with Permafluor V scintillator system (Packard) for measurement of radioactivity.

**Separation and Identification of Urinary Metabolites by HPLC.** Urinary metabolites were separated by HPLC, using a Chrompack (Middelburg, The Netherlands) C18 Hypersil ODS-5 reverse phase column (250 × 4.6 mm) and gradient elution, with solvent A consisting of 0.2% TFA in water and solvent B consisting of 0.2% TFA in acetonitrile. The following gradient program was used: from 0 to 6 min, isocratic elution at 100% A/0% B; from 6 to 11 min, gradient to 90% A/10% B; from 11 to 22 min, gradient to 0% A/100% B; from 22 to 25 min, isocratic elution at 0% A/100% B; from 25 to 27 min, gradient back to 100% A/0% B. The flow rate of the mobile phase was 1 ml/min. Urine samples were centrifuged at 12,000g before injection (100 μl). The HPLC system used consisted of a Pharmacia LKB autosampler 2157 automatic injector (temperature controlled at 4°C), a Spectra type 200 gradient pump, an LDC analytical UV detector, and an in-line Canberra Packard Radioactive detector (type A500, Flo-one beta). A liquid flow cell of 500 μl was used, and radioactivity was measured using Flo Scint A scintillation fluid (Packard) delivered at 2 ml/min.

For identification of metabolites by GC/MS, a urine sample (1 ml) obtained from an animal dosed with unlabeled 1,2-DBE (80 mg/kg) was fractionated using the same HPLC conditions as described above. Fractions containing the individual peaks observed in the HPLC chromatogram (i.e. TDA-50, TDA, 2-HMA, and M1 through M6, as indicated in fig. 5) were collected. A total of 10 runs were performed, and the corresponding fractions were pooled. The pooled HPLC fractions were divided into two equal parts, of which one was reduced with TiCl3 (see above) and the other was used for direct extraction of products.

**LC/MS.** LC/MS experiments were performed with a TSQ 7000 instrument (Finnigan MAT, San Jose, CA) equipped with either an atmospheric-pressure chemical ionization interface or an electrospray ionization interface. Typical conditions for the atmospheric-pressure chemical ionization interface were as follows: corona, 5 μA; vaporizer, 500°C; capillary, 180°C; sheath gas pressure, 70 psi; auxiliary gas pressure, 10 psi; electron multiplier setting, 1500 V. Typical conditions for the electrospray ionization interface were as follows: spray voltage, 1 kV; capillary, 200°C; sheath gas pressure, 70 psi; auxiliary gas pressure, 10 psi; electron multiplier setting, 1500 V. Full-scan data acquisition was performed over the mass range of 50–750 amu, at 1 scan/sec.

The HPLC system used was a Waters (Milford, MA) LC system, consisting of a LC pump (model 616) connected to a controller (model 600 S) and an autosampler (model 717). The column, mobile phase, and gradient program were the same as described above for the other HPLC measurements.

**Identification of Urinary Metabolites by GC/MS.** GC/MS measurements were performed with urine samples, urine fractions collected from HPLC, and TiCl3-reduced urine samples after acidification and extraction with ethyl acetate as described before (Wormhoudt et al., 1997). Briefly, urine samples (0.5 ml) were acidified by addition of 75 μl of 2 M HCl, after which they were extracted two times with 3 ml of ethyl acetate. The ethyl acetate layers were combined and evaporated under a gentle stream of nitrogen. The dry residues were dissolved in 300 μl of methanol, after which they were methylated by addition of an excess of an ethereal solution of diazomethane for at least 30 min. The solvents and excess diazomethane were evaporated under a gentle stream of nitrogen, and the dry residues were dissolved in 200 μl of ethyl acetate before analysis by GC. One-half of the volume of the collected HPLC fractions was also used for extraction and subsequent GC/MS measurements. The volume of these fractions was reduced to approximately 0.5 ml by evaporation under a gentle stream of nitrogen (at room temperature), after which the fractions were acidified and extracted in the same way as the original urine samples. The TiCl3-reduced urine samples and the urine fractions were diluted with 2 volumes of water, after which they were extracted with ethyl acetate and treated as described above.

GC/MS measurements were performed with a Hewlett Packard 5890 series II gas chromatograph coupled to a Hewlett-Packard 5970 mass-selective detector, using electron impact ionization (electron energy, 70 eV). A CPSi5CB column (Chrompack), with a length of 50 m, an internal diameter of 0.25 mm, and a stationary phase thickness of 0.2 μm, was used. The oven was programmed from 50°C (1 min) to 288°C, at a rate of 30°C/min. The temperatures of the injection port and detector were 250°C. The retention times of anticipated metabolites and the ions used for selected-ion monitoring analysis of urine samples using the described GC/MS method are indicated in table 1.

**Enzymatic Hydrolysis of Metabolites in Urine Samples.** To detect possible glucuronon conjugates, 500 μl of urine was mixed with 500 μl of 0.1 M sodium acetate buffer (pH 7), after which 100 μl of a β-glucuronidase solution was added. For the determination of sulfate conjugates, 500 μl of urine was mixed with 500 μl of 0.1 M sodium acetate buffer (pH 5), after which 100 μl of a mixture of
β-glucuronidase and arylsulfatase from H. pomatia was added. These mixtures were incubated for 20 hr at 37°C. Control incubations were performed with mixtures of the urine samples with buffer but without the enzymes.

**Data Analysis.** When average values are given, the variability is indicated by the SD.

**Results**

**Measurement of Radioactivity in Urine, Feces, Expired Air, and Tissues.** 1,2-[^14]CDBE was administered to rats either orally (group A, 50 mg/kg; group B, 150 mg/kg) or iv (group C, 10 mg/kg; group D, 50 mg/kg). Subsequently, the amounts of radioactivity were determined in urine, feces, expired air, and tissues. The cumulative amounts of radioactivity excreted in urine after 168 hr were 82.1 ± 2.8% and 80.4 ± 2.6% of the dose for the rats given orally administered 1,2-[^14]CDBE (groups A and B, respectively) and 81.1 ± 3.2% and 74.7 ± 1.9% of the dose for the rats given iv administered 1,2-[^14]CDBE (groups C and D, respectively) (fig. 1). Most of the radioactivity was excreted within 48 hr. The amounts of radioactivity excreted in expired air after 168 hr were 0.5 ± 0.2% and 0.8 ± 0.4% of the dose for the rats given orally administered 1,2-[^14]CDBE (groups A and B, respectively) and 6.0 ± 0.3 and 7.2 ± 0.2% of the dose for the rats given iv administered 1,2-[^14]CDBE (groups C and D, respectively) (fig. 3). The amounts of radioactivity in expired air were thus clearly higher in the animals dosed iv, compared with those dosed orally. Most of the radioactivity measured at 168 hr after administration of 1,2-[^14]CDBE in the organs was present in the liver, lungs, and kidneys (together <1% of the dose) and red blood cells (0.3% of the dose) (fig. 4).

**Separation and Identification of Urinary Metabolites by HPLC.** Urine samples were separated by reverse phase HPLC, in which nine metabolites were identified. A typical HPLC chromatogram of a urine sample showing these metabolites is shown in fig. 5. Synthetic standards of TDA-SO, TDA, and 2-HEMA coeluted with the peaks observed at 3.90, 11.70, and 14.4 min, respectively. Standards of 2-bromoethanol and 2-bromoacetic acid did not coelute with any of the observed 1,2-DBE metabolites. Treatment of urine samples with β-glucuronidase or the H. pomatia enzyme mixture (containing both β-glucuronidase and arylsulfatase) did not lead to any change in the HPLC chromatograms. Furthermore, no unchanged 1,2-DBE was found in the urine samples. Despite several attempts to detect 2-GEMA using LC/MS, this compound, which was once reported to...
be a trace metabolite of 1,2-DBE (Kim and Guengerich, 1989), could not be identified in the urine samples. In addition to the three identified metabolites (2-HEMA, TDA, and TDA-SO), six others, referred to as M1 through M6, were observed (fig. 5). The peaks labeled M4 and M6 were observed consistently in the urine samples collected between 8 and 24 hr for group D (dosed with 50 mg/kg 1,2-DBE iv) but not in any of the other urine samples. To further characterize the urinary 1,2-DBE metabolites, a urine sample obtained from an animal that had been given orally administered unlabeled 1,2-DBE was fractionated by HPLC. The collected fractions were subsequently used for GC/MS analysis.

**Identification of Urinary Metabolites by GC/MS.** GC/MS analysis was performed with ethyl acetate extracts of a urine sample and of urine fractions collected from HPLC (a urine sample from an animal that had been dosed orally with 80 mg/kg unlabeled 1,2-DBE was used). TDA and 2-HEMA were identified in the fractions containing the HPLC peaks eluting at 11.70 and 14.40 min, respectively. TDA could even be measured in the full-scan mode and yielded fragments at \( m/z \) 178 (molecular ion, 20.8%), 146 (72.6%), 118 (65%), and 119 (79.2%), at a retention time of 7.54 min, corresponding to data previously reported (Wormhoudt et al., 1997) and data obtained with the corresponding reference compound. 2-HEMA was also detected, at a retention time of 11.40 min, by selected-ion monitoring using fragments at \( m/z \) 203, 162, and 144 (table 1). No MS evidence could be obtained for the presence of CM-MA, HE-TA, the pyruvic acid conjugates CM-PYR and HE-PYR, or the lactic acid conjugates CM-LACT and HE-LACT (fig. 6) in an extracted urine sample after GC/MS analysis, using selected-ion monitoring of fragments observed for the reference compounds (table 1).

Urine samples and the urine fractions collected from HPLC were also subjected to reduction with TiCl\(_3\) in HCl, to reduce possible sulfoxides present. The HPLC fraction containing the metabolite with a retention time of 3.9 min contained TDA after reduction with TiCl\(_3\) in HCl, whereas before reduction no TDA was detectable in this HPLC fraction. This confirms the identity of this metabolite as TDA-SO. Urine fractions from nontreated rats did not contain TDA or TDA-SO.

No evidence was obtained for the presence of CM-MA, HE-TA, the pyruvic acid conjugates CM-PYR and HE-PYR, or the lactic acid conjugates CM-LACT and HE-LACT in a TiCl\(_3\)/HCl-reduced and ethyl acetate-extracted urine sample after GC/MS analysis, using selected-ion monitoring of characteristic fragments observed for the

![Fig. 2. Cumulative excretion of radioactivity in the feces.](image)

The cumulative excretion of radioactivity in the feces is presented individually for all four groups of animals given 1,2-[\(^{14}\)C]DBE, at a dose of 50 mg/kg orally (A), 150 mg/kg orally (B), 10 mg/kg iv (C), or 50 mg/kg iv (D). The symbols used to represent the data for the individual animals are the same as in fig. 1.
corresponding reference compounds (table 1). In the case of 2-HEMA, HE-TA, and HE-LACT, chlorination of the hydroxyethyl groups was observed after reduction of the reference compounds with TiCl₃/HCl; however, none of these chlorinated analogues was found in any of the urine samples. These results indicate that no sulfoxides of these anticipated GSH-derived metabolites were present in urine from 1,2-DBE-treated rats.

Quantification of Urinary Metabolites. The cumulative amounts of the individual metabolites excreted in urine are given in table 2. TDA was excreted in amounts between 9.2 and 13.6% of the dose in the four different groups of rats. The route of administration of the dose did not significantly influence the excretion of TDA. TDA-SO was excreted in amounts about 2-fold higher, compared with TDA, in groups A, B, and C but in almost equal amounts in group D. 2-HEMA was excreted at 22–25% of the dose in groups A, C, and D and 34% of the dose in group B. The three metabolites 2-HEMA, TDA, and TDA-SO accounted, on average, for 78% of radioactivity in the urine samples. Based on the excretion rates for these three metabolites in urine, elimination half-lives were calculated for the groups of rats administered 1,2-DBE iv (groups C and D). In group C, 2-HEMA had an elimination half-life of 6.3 ± 1.8 hr, TDA one of 7.0 ± 4.3 hr, TDA-SO one of 8.4 ± 2.1 hr, and the sum of TDA and TDA-SO one of 6.9 ± 1.0 hr. In group D, the values were 11.8 ± 5.9, 4.9 ± 1.4, 7.8 ± 1.1, and 6.8 ± 1.0 hr for 2-HEMA, TDA, TDA-SO, and the sum of TDA and TDA-SO, respectively. The elimination half-life for the sum of TDA and TDA-SO was calculated because TDA-SO is a secondary metabolite of TDA.

Discussion

The aim of the present study was to investigate the disposition of 1,2-DBE in rats. The results of this study might be valuable for risk assessment and biomonitoring studies of 1,2-DBE and for the validation of a recently developed PBPK model for 1,2-DBE in rats and humans (Ploemen et al., 1997). This PBPK model includes individual variability in biotransformation in human risk assessment for this carcinogenic compound.

Cumulatively, 82.1 ± 2.8 and 80.4 ± 2.6% of the 1,2-DBE dose was recovered in urine from rats given orally administered 1,2-DBE (50 and 150 mg/kg, respectively), with 81.1 ± 3.2 and 74.4 ± 1.9% in urine from rats given iv administered 1,2-DBE (10 and 50 mg/kg, respectively); most of the dose was excreted within 48 hr after dosing. In a previous study in guinea pigs, a somewhat smaller amount of 66%
of a 30 mg/kg dose of 1,2-DBE administered i.p. was recovered in urine within 72 hr (Plotnick and Conner, 1976). The amounts of radioactivity in expired air were clearly dependent on the route of administration. The rats given orally administered 1,2-DBE (50 and 150 mg/kg) exhaled 0.5 ± 0.2 and 0.8 ± 0.4% of the dose, whereas the rats given i.v. administered 1,2-DBE (10 and 50 mg/kg) exhaled 6.0 ± 0.3 and 7.2 ± 0.2% of the dose, respectively. The greater amounts of radioactivity in the expired air in the case of iv administration might be explained by an earlier and faster exchange between blood and air in the lungs in the case of iv administration, compared with oral administration. The amounts of radioactivity recovered from the tissues were low, most likely because of the fact that these were only measured 168 hr after administration of 1,2-DBE. Most of the radioactivity was found in the liver, lungs, and kidneys (together <1% of the dose) and red blood cells (0.3% of the dose). Because these measurements were made only after 168 hr, it is difficult to relate the covalent binding of 1,2-DBE in these tissues to its toxicity, which is observed in a number of organs (Alexeeff et al., 1990).

The urinary metabolites of 1,2-DBE identified in the present study are 2-HEMA, TDA, and TDA-SO. 2-HEMA was reported previously to be a metabolite of 1,2-DBE and was excreted as 30–55% of orally administered 1,2-DBE doses between 10 and 100 mg/kg (van Bladeren et al., 1980, 1981) and as 41 and 24% of i.p. administered doses of 10 and 20–40 mg/kg, respectively (Wormhoudt et al., 1997). The excretion of 2-HEMA in the present study was comparable, being 23.6 ± 1.3% of the dose in rats given 50 mg/kg orally or 10 or 50 mg/kg i.v. and 33.8 ± 0.7% of the dose in rats given 150 mg/kg orally. Because the P450-mediated metabolic pathway seems to be saturated in rats administered 150 mg/kg 1,2-DBE orally (no more TDA was excreted in urine in this group, compared with the groups administered lower doses of 1,2-DBE), the increased amount of 2-HEMA excreted in group B is likely to be formed via the nonsaturated GST-mediated pathway of 1,2-DBE metabolism. It must be noted that the P450-mediated oxidation of 1,2-DBE is only the first step in the formation of TDA and more enzymes are involved, which might be saturated as well. The potential saturation of the P450 route of metabolism has implications for the risk of exposure to 1,2-DBE, because at high exposure levels the nonsaturated GST-mediated route of metabolism, which is thought to be responsible for the genotoxicity of 1,2-DBE, would become more important. Previously, we showed that the GST-mediated metabolism of 1,2-DBE in human erythrocytes
is influenced by the genetic polymorphism of human β-class GSTs (Ploemen et al., 1995).

TDA was recently reported to be a P450-specific metabolite of 1,2-DBE and was excreted as, on average, up to 20% of ip administered 1,2-DBE doses between 10 and 40 mg/kg and 13.1% of orally administered doses of 40 mg/kg (Wormhoudt et al., 1997). The amounts of TDA found in the present study, i.e. between 9.2 and 13.6% of the dose, compare well with those previously measured.

For several other compounds, it was reported that TDA-SO was detected in urine in addition to TDA itself, with TDA-SO in those cases representing between 67 and 100% of the amount of TDA (Brakenhoff et al., 1993, Hofmann et al., 1991; Godeneche et al., 1993). In the present study, the amount of TDA-SO was equal to (group D) or approximately 2-fold greater than (groups A, B, and C) that of TDA itself.

The elimination half-lives of 2-HEMA, TDA, and TDA-SO calculated for the rats given 1,2-DBE iv were not significantly different for the two administered doses of 1,2-DBE. Furthermore, they were longer than the elimination half-life for 1,2-DBE elimination from plasma in rats, which is between 15 and 120 min, depending on the dose and route of administration.

The boxed compounds 2-HEMA, TDA, and TDA-SO were unequivocally identified as major metabolites of 1,2-DBE in the present study. In contrast, the other indicated metabolites were not found as 1,2-DBE metabolites. This metabolic scheme points toward a difference in the metabolism of the intermediates CMC and 2-HEC, because CMC is ultimately converted to TDA (and TDA-SO) and 2-HEC is primarily acetylated to 2-HEMA. Additional details can be found in the text.

**Fig. 6.** Proposed schematic representation of the metabolism of 1,2-DBE.

This scheme shows the proposed metabolism of 1,2-DBE. The boxed compounds 2-HEMA, TDA, and TDA-SO were unequivocally identified as major metabolites of 1,2-DBE in the present study. In contrast, the other indicated metabolites were not found as 1,2-DBE metabolites. This metabolic scheme points toward a difference in the metabolism of the intermediates CMC and 2-HEC, because CMC is ultimately converted to TDA (and TDA-SO) and 2-HEC is primarily acetylated to 2-HEMA. Additional details can be found in the text.

Based on the positively identified urinary metabolites and several excluded urinary metabolites, the metabolic profile of 1,2-DBE shown schematically in fig. 6 can be proposed. Upon oxidation of 1,2-DBE by P450 enzymes, 2-BA is formed, which can be conjugated with GSH to form S-(2-oxoethyl)glutathione. This GSH conjugate can be either oxidized or reduced. Oxidation gives rise to formation of S-(carboxymethyl)glutathione and subsequently the cysteine conjugate CMC. Several metabolic reactions are possible with this cysteine conjugate (for a review of these reactions, see Commandeur et al., 1995). CMC can be acetylated to form CM-MA but might also be metabolized further by deamination to a thiopyruvic acid conjugate, CM-PYR, which is rapidly reduced to CM-LACT or decarboxylated to TDA. Furthermore, all of these potential metabolites can be sulfoxidated. CM-LACT was reported previously as a metabolite of CMC in humans, as 2.1% of the CMC dose (for comparison, 33.1% of the CMC dose was recovered as the sum of TDA and TDA-SO) (Hofmann et al., 1991). That finding prompted us to search for CM-LACT in urine of rats given 1,2-DBE, but this metabolite could not be detected. Furthermore, CM-MA was not found in the urine samples. Reduction of S-(2-oxoethyl)glutathione leads to the formation of S-(2-hydroxyethyl)glutathione and the corresponding cysteine conjugate 2-HEC. The same kind of metabolism as described for CMC can be proposed for 2-HEC. Thus, 2-HEC can be acetylated to 2-HEMA, which was indeed identified as one of the major metabolites of 1,2-DBE in the present study. Theoretically, 2-HEC might also be metabolized to the thiopyruvic acid conjugate HE-PYR, the thiolactic acid conjugate HE-LACT, and HE-TA. However, HE-PYR, HE-LACT, and HE-TA or their sulfoxides could not be detected as urinary metabolites.
metabolites of 1,2-DBE in the present study. These findings thus indicate a major difference between the metabolism of CMC and that of 2-HEC; CMC is ultimately converted to TDA, whereas 2-HEC is predominantly acetylated to form 2-HEMA.

Upon GST-catalyzed metabolism of 1,2-DBE, a reactive episulfonium ion is formed, which may be hydrolyzed to form (2-hydroxyethyl)glutathione but which may also react with guanine bases in the DNA and ultimately be converted into the mercapturic acid 2-GEMA. Although 2-GEMA was once reported to be a trace metabolite of 1,2-DBE (Kim and Guengerich, 1989), our attempts to identify 2-GEMA in rat urine by LC/MS were unsuccessful.

In a previous study on the metabolism of 1,2-DBE in rats, three major metabolites were detected in urine GC with sulfur-selective detection (Wormhoudt et al., 1997). Two of these metabolites were identified as 2-HEMA and TDA, whereas the identity of the third metabolite was not established. Based on the response of the unknown compound in the sulfur-selective detector, it was thought to be a quantitatively important metabolite, probably formed by a GSH-dependent route of metabolism. However, we could not identify any new GSH-derived metabolites in the present study, and none of the GSH-derived metabolites investigated exhibited a retention time comparable to that of the unknown metabolite in GC with sulfur-selective detection (data not shown). Therefore, the identity of the previously detected unknown metabolite still remains to be established.

In conclusion, comprehensive and relevant information on the disposition of 1,2-DBE was obtained in this study. 2-HEMA, TDA, and TDA-SO were identified as the major urinary metabolites of 1,2-DBE, together accounting for 78% of the recovered radioactivity in urine. However, the identities of several unidentified minor metabolites of 1,2-DBE remain to be established. The data obtained might be valuable for risk assessment and biomonitoring studies of 1,2-DBE and will also be used for further validation of a PBPK model that we recently developed for 1,2-DBE in rats and humans (Ploemen et al., 1997). This PBPK model includes interindividual variability in bio-transformation in human risk assessment for this carcinogenic compound.

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