Misuse of diethylene glycol (DEG) has led to numerous epidemic poisonings worldwide. DEG produces toxicity because of its metabolism, although the mechanism of its toxicity has not been further defined. The purpose of this study was to investigate the accumulation of specific metabolites in blood and target organ tissues and to determine the relationship between tissue accumulation of metabolites and the resulting toxicity. Wistar rats were treated with water, 2 g/kg DEG (low dose), 10 g/kg DEG (high dose), or 10 g/kg DEG + fomepizole (15 mg/kg then 10 mg/kg per 12 h, to inhibit DEG metabolism), and renal and tissue samples were collected up to 48 h. After high doses of DEG, 2-hydroxyethoxyacetic acid (HEAA) was the primary metabolite in the blood (4 mmol/l), with only low concentrations of diglycolic acid (DGA) (~0.04 mmol/l). In contrast, renal and hepatic concentrations of DGA and of HEAA at 48 h were similar (~4 mmol/l), indicating a 100-fold concentrative uptake of DGA by kidney tissue. Treatment with fomepizole blocked the formation of HEAA and DGA and the kidney toxicity. Both HEAA and DGA concentrations in the kidney correlated strongly with the degree of kidney damage. Accumulation of HEAA in blood correlated with increased anion gap and decreased blood bicarbonate so appeared responsible for the DEG-induced acidosis. Although these studies suggest that either metabolite may be involved in producing kidney toxicity, the unexpected renal accumulation of DGA at toxic doses of DEG suggests that it must also be considered a possible toxic metabolite of DEG.

Key Words: diethylene glycol; fomepizole; nephrotoxicity; metabolic acidosis; hydroxyethoxyacetate; diglycolic acid; oxybisacetate.

Diethylene glycol (DEG), although used primarily as an industrial chemical (solvent, lubricant, or chemical intermediate), is also found in various automotive products, which thus can lead to potential consumer exposure and poisoning episodes (Marraffa et al., 2008). Illicit or mistaken use of DEG as a pharmaceutical solvent has resulted in numerous epidemic poisonings since the 1930’s (Barr et al., 2007), including Panama in 2006 (Schep et al., 2009) and Nigeria in 2008 (Abubukar et al., 2009). Though nearly a 1000 people have died worldwide in epidemic DEG poisonings, children are especially vulnerable to the toxicant due to the need to formulate medications that are often administered to them. At least five epidemic DEG poisonings have targeted children and have resulted in over 300 deaths (Alkahtani et al., 2010), including Haiti in 1995 (O’Brien et al., 1998), India in 1986 and 1998 (Hari et al., 2006; Pandya, 1988; Singh et al., 2001), and in Nigeria in 1990 and 2008 (Abubukar et al., 2009; Okuonghae et al., 1992). Because of the lack of effective diagnostic procedures and treatment strategies, the morbidity and mortality in DEG poisonings is remarkable (Barr et al., 2007). The major clinical manifestations of DEG poisoning, including metabolic acidosis, renal failure, and peripheral neuropathy, are well known (Alfred et al., 2005; O’Brien et al., 1998). Although acute DEG toxicity involves effects on multiple organ systems, the hallmark of its toxicity is development of acute renal failure.

Despite the wealth of clinical knowledge regarding DEG toxicity, the mechanisms for its toxicity are not fully understood. Recently, DEG toxicity has definitively been shown to result from its metabolism via alcohol dehydrogenase (ADH) because inhibition of ADH by fomepizole prevented the development of renal failure (Besenhofer et al., 2010). In those studies, fomepizole was used at doses (10–15 mg/kg) that primarily inhibited ADH and did not affect the activity of cytochrome P450 2E1 (Chow et al., 1992), which theoretically might also oxidize DEG. Although metabolites of DEG have been reported in the urine of DEG-treated animals, no studies have reported on the accumulation of metabolites in the target organ (kidney) or in the blood. As such, the metabolite of DEG that is responsible for the kidney toxicity has yet to be determined. Tracer studies using nontoxic doses in rats and dogs (0.5–5 g/kg of [14C]-DEG) reported that 70–80% of the dose...
was excreted in the urine as unchanged DEG and 10–30% as urinary 2-hydroxyethoxycetic acid (HEAA) (Lenk et al., 1992; Wiener and Richardson, 1989). No other labeled forms were reported in these studies. After toxic doses of DEG in rats (10 g/kg of unlabeled DEG), unchanged DEG and HEAA were also the major urinary metabolites (Bessenhofer et al., 2010). However, that study reported, for the first time, the presence of low concentrations of diglycolic acid (DGA) in the urine, apparently a result of further oxidation of HEAA by unknown pathways. The ethylene glycol (EG) metabolites glycolic acid (GA) and oxalic acid (OA) were not detected in any of these studies. In DEG-intoxicated patients, oxalate crystals have also not been found in the urine or kidney tissues (Alfred et al., 2005; Brophy et al., 2000; Okungahwe et al., 1992). Thus, the renal toxicity from DEG does not result from oxalate formation but instead from the renal accumulation of either HEAA or DGA.

At present, there are no good therapies for treating DEG-intoxicated patients because of the lack of knowledge about its mechanism of toxicity. Antidotal treatment with the ADH inhibitor fomepizole could be useful to prevent target organ toxicity in acute DEG poisonings, providing a diagnosis is made quickly and aggressive treatment is begun. However, this treatment would not be useful for late-diagnosed poisonings (such as in mass epidemics) because the latter would already result in accumulated metabolites and toxicity. Because exposure to DEG is likely in the United States as well as worldwide and because a target population is children, understanding the toxic mechanisms of DEG is critical in order to better diagnose and treat DEG poisoning. The first mechanistic step would logically be to identify the toxic metabolite. Although HEAA and DGA have been identified in the urine of DEG-intoxicated rats, their accumulation in the blood or target organ tissues has yet to be linked with the observed toxicity. The purpose of this report was to characterize the accumulation of toxic metabolites during acute DEG intoxication and to relate accumulation to development of toxicity, which would allow for subsequent studies of toxic mechanisms in vivo.

MATERIALS AND METHODS

Materials. DEG, provided by Shell Chemicals, was analyzed for purity by gas chromatography (GC) with flame ionization detection prior to dosing. The DEG contained DEG (99.78%), EG (0.05%), and triethylene glycol (0.08%). Synthetic standards of DEG and OA, with chemical purities of 100 and 99.6%, respectively, were obtained from Fluka Chemical Corporation (Milwaukee, WI). Standards of EG, DGA, and GA with chemical purities of 99.8, 99.4, and 100%, respectively, were obtained from Sigma-Aldrich Corporation (St Louis, MO). A standard of HEAA with a chemical purity of 99% was obtained from Isotec Incorporated (Miamisburg, OH). The internal standards (g//ml for HEAA, DGA, and OA in liver tissue, respectively, and 13C2-OX were all obtained from Isotec Incorporated. 4-Methylpyrazole (4-MP, 99%, from Sigma-Aldrich) was used for these studies. Because fomepizole, the generic pharmaceutical in the United States, is the same formulation (free base 4-MP), the term fomepizole is used in the manuscript for its clinical relevance.

Animal protocol. Adult male Wistar rats (425–475 g; Harlan, Indianapolis, IN) were implanted with chronic indwelling jugular catheters and allowed to recover as described previously (Bessenhofer et al., 2010). Animals were randomly placed into four treatment groups (n = 6 per group) including 10 g/kg DEG, 2 g/kg DEG, water, or 10 g/kg DEG plus fomepizole. DEG was administered by oral gavage at time 0, and fomepizole was administered ip as a 10 mg/ml solution in saline at 15 min (15 mg/kg) and then as 10 mg/kg at 12, 24, and 36 h. Animals were housed in metabolic cages for 48 h for urine collection following an acclimation period of 8–10 h. Standard conditions of humidity (50 ± 10%), temperature (22°C ± 2°C), and light (12:12-h light:dark) were maintained in the animal room, and all rats were allowed free access to food (normal rat chow) and water. The animal protocols were approved by the Institutional Animal Care Committee (Louisiana State University Health Sciences Center, Shreveport, LA) and were in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

Urine collection and analysis. Urine was collected at 4, 8, 12, 24, 36, and 48 h over ice to minimize degradation of urinary metabolites. Metabolic cages were rinsed with deionized water between collections. Immediately after collection, the urines were vortexed and the volume was recorded. Two 1-ml aliquots of vortexed urine were transferred to cryogenic vials and stored at –80°C until analysis.

Blood collection and analysis. Blood (~1 ml) was collected via the indwelling jugular catheter at 4, 8, 12, 24, 36, and 48 h into heparinized syringes and immediately placed on ice. Heparinized whole blood was analyzed for pH, pCO2, and pO2 by the Radiometer ABL800 FLEX blood gas analyzer; blood bicarbonate concentrations were calculated by the analyzer. The remaining blood was transferred to separator tubes (Ref 365956; BD, Franklin Lakes, NJ), inverted, and centrifuged at 12,000 × g for 5 min to isolate the plasma, which was stored at 4°C until analysis. Plasmas were analyzed on the Abbott Architect Ci8200 for a basic metabolic panel by the LSUHSC-S Clinical Laboratory including concentrations of electrolytes Na+, K+, Cl–, and plasma CO2. The anion gap was calculated from the plasma electrolyte and CO2 values.

Tissue collection. At 48 h, the animals were anesthetized with sodium pentobarbital (50 mg/kg, ip) and the kidney and liver were collected for metabolic analysis. One kidney was cut in half and one half was placed in foil and immediately put onto dry ice. A lobe of the liver was similarly removed and frozen on dry ice. After freezing was complete, the foiled tissue was placed in a labeled tube that was stored at –80°C until metabolite analysis. The remaining kidney and liver were preserved for histopathology evaluation and the results are presented elsewhere (Bessenhofer et al., 2010).

Determination of metabolite concentrations in blood and tissues. DEG and EG in blood or tissues were determined by an existing method that employs chemical derivatization with pentfluorobenzoyl chloride followed by separation with GC and detection by negative chemical ionization mass spectrometry (MS) (Pottinger et al., 2001), with a few modifications, primarily in sample preparation. A complete description of this modified method is included as Supplementary information. The achieved limits of quantitation (LOQ) were 5 µg/ml for each analyte in blood and 2.1 and 5.1 µg/ml for DEG and EG, respectively, in tissues.

The potential acid metabolites (OA, GA, DGA, and HEAA) were determined in blood and tissues using ion chromatography (IC) with detection by negative electrospray ionization MS. These methods are based on those previously used for these compounds in the urine (Bessenhofer et al., 2010) but with modifications for sample preparation and some separation and MS detection parameters. A complete description of these modified methods is included as Supplementary information. The achieved LOQ were 1.9, 2.2, 32, and 9.2 µg/ml for HEAA, DGA, GA, and OA in liver tissue, respectively, and 2.0, 2.1, 19.4, and 12.1 µg/ml for HEAA, DGA, GA, and OA in kidney tissue, respectively.
Mass balance analysis of metabolite recovery. For urine samples, the molar amount of each metabolite excreted from 0 to 48 h was determined as the sum of the amounts in each interval, calculated from the concentration and volume excreted in each interval. Percent dose was then calculated as molar amount excreted × 100/molar amount of DEG dosed. For tissue samples, the molar amount of each metabolite was determined from the tissue concentration and the tissue weight. Because each individual metabolite contributed a very small percent of dose, the total molar amount in the tissue was calculated as the sum of the four metabolites. Percent dose was then calculated as total molar amount in the tissue × 100/molar amount of DEG dosed.

In vitro analysis of crystal formation from DGA. To assess whether DGA crystallizes in the presence of calcium, solutions of DGA (20 and 40 mmol/l) were mixed equivolumetrically with solutions of calcium chloride (20 and 40 mmol/l) and then observed over a period of 48 h.

Statistics. Values in the text represent the group mean value ± SEM. Differences between treatment groups and time points were assessed with two-way ANOVA with Bonferroni post hoc test. To compare differences between treatment groups only, one-way ANOVA with Tukey post hoc test was used. All analyses were performed using GraphPad Prism 5 for Windows. Tests were considered significant if \( p < 0.05 \).

RESULTS

DEG Metabolite Levels in the Blood

The blood concentrations of HEAA, DGA, DEG, and EG are shown in Figure 1. DEG levels peaked at 4 h at 8 and 30 mmol/l after the 2 and 10 g/kg doses, respectively, indicating a near dose proportionality for the Cmax (Table 1). Higher concentrations of DEG at 8 and 12 h were observed in the rats treated with 10 g/kg and fomepizole. Analysis of the blood DEG levels for individual rats by first-order elimination kinetics was used to determine the elimination rate and half-lives for the various dose groups (Table 1). The elimination values for 10 g/kg + fomepizole group were not significantly different from that for the 10 g/kg group.

Blood HEAA concentrations in the 10 g/kg group peaked at 4.2 mmol/l at 8 h after the DEG dosing (Fig. 1A). The HEAA levels declined to about 24 h, where they appeared to plateau. At these later time points, there was high variability among individual rats. Out of the six rats in this group, two showed renal failure by 36 h (Besenhofer et al., 2010), whereas the others showed variable renal damage but not failure. The HEAA levels in the animals without failure declined from 24 to 48 h, whereas those in the two rats with failure increased dramatically to ~13 mmol/l by 48 h. Lesser amounts of HEAA were detected in the blood after the low dose of DEG, peaking at 4 h and rapidly declining thereafter. Blood HEAA levels in the 10 g/kg + fomepizole group were not significantly different from those in control animals.

Blood DGA concentrations were increased only in the high-dose group (Fig. 1B), to a peak at 24 h of 0.04 mmol/l, or 100-fold less than the highest HEAA concentration. Similarly to HEAA, there was a large variability in blood DGA levels at 36 and 48 h. Levels in the rats that had renal failure increased markedly to reach 0.2 mmol/l at 48 h. Blood DGA levels in the 10 g/kg + fomepizole group were not significantly different from those in control animals.

EG levels in the blood also increased after high-dose DEG administration (Fig. 1D), to a peak at 12 h of 0.33 mmol/l (about 2 mg/dl). These EG concentrations do not result in any increase in GA or OA in the blood (data not shown). The blood levels of EG after the high dose of DEG were reduced by fomepizole, being significantly lower at 8, 12, and 24 h in the 10 g/kg + fomepizole rats than in the 10 g/kg group.

DEG Metabolite Levels in the Kidney and Liver

Kidney and liver levels of DEG and its metabolites were determined in tissue samples collected at the end of the study (48 h). Figures 2 and 3 show the metabolite levels in the kidney and liver, respectively. In general, increases in the kidney and liver levels of the four metabolites (DEG, HEAA, DGA, and EG) were observed only in the 10 g/kg group. At this toxic dose of DEG, the kidney concentrations of HEAA and DGA were almost identical, both occurring at about 4–5 \( \mu \)g/organ. If 1 g of kidney tissue is considered roughly equivalent to 1 ml, then the kidney HEAA concentrations were approximately 4–5 mmol/l, which are thus similar to the peak blood levels of HEAA (Fig. 1). However, the kidney levels of DGA were about 100-fold higher than the peak DGA levels in the blood (Fig. 1), demonstrating substantial accumulation of DGA in the kidney. Kidney and liver concentrations of DEG and of EG were lower than those of the two acid metabolites, representing about 1 and 0.1 \( \mu \)g/organ, respectively. No GA was detected in either tissue, and the levels of OA in the tissues in the high-dose DEG group were not elevated over those in the control group (data not shown). Tissue concentrations of the acid metabolites, HEAA and DGA, were negligible after the low dose of DEG (2 g/kg) and in the 10 g/kg + fomepizole group.

Correlation of Kidney Levels of Metabolites with Toxicity

The renal toxicity biomarkers, blood urea nitrogen (BUN) and plasma creatinine, were elevated in the high-dose DEG-treated rats at 36 and 48 h (Besenhofer et al., 2010). The rise in BUN and creatinine correlated well with the tissue levels of HEAA (Fig. 4A) and moderately well with the tissue levels of DGA (Fig. 4B), determined at 48 h. Neither parameter correlated well with the peak urine concentrations of HEAA or DGA nor with the peak blood levels of either metabolite (data not shown).

In Vitro Analysis of Crystal Formation from DGA

DGA is a dicarboxylate anion with structural similarity to OA, which readily forms crystals in the presence of calcium. To assess the likelihood that DGA might also crystallize in the presence of calcium, solutions of DGA and of calcium chloride were mixed as described in “Materials and Methods” section. Observation for 48 h showed no crystal formation—under these circumstances, calcium oxalate precipitates immediately.
Correlation of Metabolites with Metabolic Acidosis

DEG, even at the high dose, did not alter the plasma concentrations of K\(^+\), Na\(^+\), and Cl\(^-\) (data not shown), electrolytes that were measured in order to calculate the anion gap. Data in Figure 5 show that the anion gap was increased by the high-dose DEG from 17 mmol/l to a maximum of 25 mmol/l at 4 h, with a decline to control levels by 24 h. The anion gap was not significantly increased at the low dose of DEG nor in the rats treated with 10 g/kg fomepizole.

Data in Figure 6 show that there was a strong correlation between the blood level of HEAA and the increase in the anion gap (\(r = 0.71\)) and an even stronger correlation with the decrease in the blood bicarbonate concentration (\(r = 0.88\)). Blood bicarbonate concentrations were significantly decreased after the high dose of DEG compared with controls (Fig. 7A). When the blood bicarbonate and blood HEAA concentrations were added together, there were no significant differences between controls and the high-dose DEG-treated rats (Fig. 7B).

Mass Balance Analysis of DEG Metabolism

From the concentrations of the various metabolites in the tissues as well as the tissue weights, the amount of the administered dose of DEG, recovered as the separate metabolites in the kidney and the liver, was calculated. Because the percent of dose recovered as an individual metabolite in either the liver or the kidney was exceedingly small, the tissue recovery of all four metabolites was combined for presentation in Table 2. From the concentrations of these metabolites in the urine (Besenhofer et al., 2010) and the urinary volume at each collection time, the amount of each metabolite recovered in the urine was also calculated. The percent of the dose recovered as each metabolite in the urine is also presented in Table 2. At the high dose of DEG, nearly 60% of the dose was found as unchanged DEG in the urine, about 25% as HEAA, with less

### Table 1: Kinetic Parameters for DEG in Male Wistar Rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>Tmax. (h)</th>
<th>Cmax (mmol/l)</th>
<th>Ke</th>
<th>T1/2 (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 g/kg DEG</td>
<td>4.0 ± 0.0</td>
<td>8.0 ± 1.0</td>
<td>0.14 ± 0.02</td>
<td>5.3 ± 0.9</td>
</tr>
<tr>
<td>10 g/kg DEG</td>
<td>4.8 ± 0.8</td>
<td>30.8 ± 5.8</td>
<td>0.06 ± 0.01</td>
<td>13.3 ± 1.9</td>
</tr>
<tr>
<td>10 g/kg DEG + fomepizole</td>
<td>6.7 ± 1.3</td>
<td>38.4 ± 4.4</td>
<td>0.07 ± 0.01</td>
<td>9.8 ± 0.9</td>
</tr>
</tbody>
</table>

Note. Values represent the mean ± SEM (n = 6, except n = 5 for 2 g/kg because there were too few blood samples in one rat to determine the kinetics).
than 1% as DGA or EG in the urine, and less than 1% as all metabolites in either the kidney or the liver. In the presence of fomepizole, nearly all the dose was excreted as unchanged DEG in the urine.

**DISCUSSION**

DEG toxicity is characterized by damage to the kidney and liver, with the kidney appearing to suffer much greater damage. In fact, acute renal failure is the hallmark of most cases of DEG intoxication (Besenhofer et al., 2010; Marraffa et al., 2008; Schep et al., 2009). Although metabolism of DEG is necessary for the production of kidney damage (Besenhofer et al., 2010), the responsible metabolite(s) have not been determined. Previous studies have identified HEAA and unchanged DEG as the primary urinary metabolites (Lenk et al., 1989; Mathews et al., 1991; Wiener and Richardson, 1989), but the present study is the first animal or human study to report on the blood concentrations of DEG metabolites and on their accumulation in target tissues. The key and surprising finding in these studies is that DGA was a major metabolite of DEG in terms of target organ retention, despite the fact that its blood and urine concentrations were relatively low. Although HEAA reached much higher peak concentrations in the blood (100 times that of DGA), DGA concentrations in kidney and liver tissue after

**FIG. 2.** Kidney HEAA (A), DGA (B), DEG (C), and EG (D) concentrations at in rats treated with DEG. Data are represented as means ± SEM (n = 6 per group).

**TABLE 2**

<table>
<thead>
<tr>
<th>Dose</th>
<th>Urine DEG</th>
<th>Urine HEAA</th>
<th>Urine DGA</th>
<th>Urine EG</th>
<th>Kidney</th>
<th>Liver</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 g/kg DEG</td>
<td>50.7 ± 3.9</td>
<td>33.1 ± 1.5</td>
<td>0.9 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>86.0 ± 3.1</td>
</tr>
<tr>
<td>10 g/kg DEG</td>
<td>57.5 ± 6.6</td>
<td>24.4 ± 2.3</td>
<td>0.4 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.3</td>
<td>83.5 ± 5.4</td>
</tr>
<tr>
<td>10 g/kg DEG + fomepizole</td>
<td>93.5 ± 2.6</td>
<td>1.3 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>95.1 ± 2.7</td>
</tr>
</tbody>
</table>

*Note.* Percent dose recovery was calculated as molar concentration × 100/molar amount of DEG dosed for each metabolite in the urine and as the sum of the four metabolites in the tissues. Values represent the mean ± SEM (n = 6).
the high dose of DEG were roughly the same as those of HEAA. Furthermore, at doses of DEG that did not produce kidney damage (2 g/kg), there was no kidney accumulation of either HEAA or DGA, suggesting that tissue retention of metabolites was important for production of toxicity.

The relatively high kidney levels of DGA occurred because there was an intriguing concentrative uptake of DGA into the kidney, with about 100-fold higher concentrations than the peak levels in the blood. In contrast, HEAA did not appear to accumulate *per se* because its peak blood and kidney levels were nearly identical. Renal clearance of HEAA is about the same as that of inulin (Young *et al.*, 1977), indicating that HEAA is filtered at the glomerulus, but then not significantly reabsorbed, which would correlate with a lack of accumulation by the kidney. Also in the blood, HEAA reached its Cmax at 8 h, whereas DGA did not peak until after 24 h. The fact that DGA remained in the blood for a longer period might help explain its long-term accumulation in the kidney. Why would the kidney tend to retain high levels of DGA? We can rule out one explanation—unlike the mechanism for oxalate accumulation in EG-treated kidneys (i.e., oxalate crystal formation and retention; Corley *et al.*, 2008; McMartin, 2009), DGA did not accumulate as kidney crystals. In the *in vitro* studies, it did not crystallize in the presence of calcium, suggesting that DGA crystals are not likely to form *in vivo*. In *vivo*, no crystal deposits were observed in the DEG-treated rat kidneys by histopathological analysis (Besenhofer *et al.*, 2010). Calcium oxalate crystals are readily observed in EG-treated rat kidneys using similar techniques (Li and McMartin, 2009) so the lack of crystals in DEG-treated rat kidneys was not a methodological issue. A potential explanation for DGA accumulation could be selective uptake by renal tubular cells. DGA is a four-carbon dicarboxylate anion that has structural similarity to a number of dicarboxylate Krebs cycle intermediates like succinate. We can speculate that DGA is a substrate for a renal sodium dicarboxylate (NaDC) transporter such as NaDC-1 or NaDC-3 (Pajor, 1999) and thus can be taken up into kidney tubular cells. Intracellular accumulation could occur if DGA were then poorly transported out of the cell. Transport of HEAA into kidney tubule cells and biotransformation to DGA within the cells could be an alternative mechanism of tissue accumulation of DGA. The transporters and enzymes responsible for this metabolism are not known.

The high degree of tissue accumulation of DGA suggests that it could be a likely cause of kidney damage, at least as likely as HEAA. It is interesting to note that a fatal accidental ingestion of

![FIG. 3.](https://academic.oup.com/toxsci/article-abstract/123/2/374/1690069)
DGA has occurred (Roscher et al., 1975) and this case appears to be the only reported human exposure in the literature. Within 24 h of ingestion, the patient reported to the hospital in acute renal failure, with elevated levels of hepatic enzyme markers. Despite repeated hemodialysis, the patient eventually succumbed. Autopsy confirmed massive renal cortical necrosis and centrilobular hepatic necrosis. Thus, in a single isolated case, DGA ingestion has produced an acute renal failure and hepatoxicity similar to that reported in DEG ingestions.

BUN values and serum creatinine levels, as markers for kidney damage (Emeigh Hart, 2005), are increased by DEG in rats and in humans (Besenhofer et al., 2010; O’Brien et al., 1998). In the present study, the kidney concentrations of both HEAA and of DGA correlated well with the increase in either BUN or plasma creatinine values. The correlations with HEAA levels appeared to be somewhat stronger, with correlation coefficients near 0.99 compared with 0.83–0.89 for DGA. As such, the present studies cannot distinguish whether HEAA or DGA is more likely to result in kidney damage, and studies of the toxicities of the two metabolites in in vitro models are needed to distinguish which metabolite is more likely responsible. Although DEG also produces a liver injury, it is well documented clinically and in animals that this toxicity is much less severe than the kidney injury. Sufficient kidney damage to induce failure is a hallmark of DEG toxicity, whereas liver damage, without failure, appears secondary. Hence, it would appear that assessing the metabolite responsible for the renal toxicity was a high priority.

Cotreatment with fomepizole to inhibit ADH (McMartin, 2010) completely prevented the accumulation of DEG metabolites (HEAA and DGA) in the blood and tissues. Interestingly, this metabolic inhibition did not markedly alter the elimination of DEG from the blood. Although blood DEG levels were slightly increased in the 10 g/kg + fomepizole-treated rats compared with the 10 g/kg rats, the elimination half-lives in the two groups were not significantly different. A reasonable explanation for these phenomena is that the rate of elimination of DEG is primarily controlled by the rate of urinary excretion of unchanged DEG rather than by its metabolism to HEAA. The fact that nearly 60% of the dose was eliminated as DEG, whereas only about 30% was eliminated.

**FIG. 4.** Kidney HEAA concentrations (A) and kidney DGA concentrations (B) correlate with the development of kidney toxicity, indicated by the BUN values (left) and the plasma creatinine concentrations (right). Peak values at the time corresponding to tissue collection (48 h) are plotted using the values from high-dose–treated rats only because other groups showed no kidney toxicity.

**FIG. 5.** High-dose DEG produces an increase in the anion gap in rats indicating development of acidosis. Anion gap calculated as \([\text{Na}^+ + \text{K}^+] - (\text{Cl}^- + \text{HCO}_3^-)\). Data are represented as means ± SEM (n = 6 per group). Asterisk (*) indicates significant difference from control rats (p < 0.05).
as metabolites (HEAA, DGA, and EG), corroborates this explanation.

Blood concentrations of both HEAA and DGA in the 10 g/kg DEG group appeared to plateau from 24 to 48 h. This plateau may reflect the variability in metabolite levels that occurs at 36 and 48 h in the 10 g/kg group. At the time, two of six rats were in complete renal failure so were likely not able to clear the metabolites—the other four rats had some degree of renal toxicity but not failure so continued to clear metabolites. Thus, the mean blood metabolite concentrations are a combination of some values that were decreasing, whereas two values were increasing greatly at the same time, thus leading to the apparent plateau.

In the rats dosed with 10 g/kg + fomepizole, nearly all the dose was recovered in the urine as unchanged DEG, indicating that our methods of urine and tissue collection and analysis were sufficient to obtain quantitative recovery of the dose. It is somewhat surprising that the total recovery of dose in rats given DEG alone reached only about 85% of the dose. Several factors could have contributed to the unexplained portion of the dose. First, elevated levels of HEAA and DGA remained in the blood at the 48-h termination, such that some of the dose could be retained in the body in tissues other than the liver and kidney. Second, tracer studies have shown that small amounts of DEG-derived radiolabel are excreted in the feces and in expired CO₂ (Mathews et al., 1991). Our studies did not quantify fecal or expired air samples, which might have contained some of the administered dose. Lastly, other metabolites of DEG, not detected in these studies, might exist. However, in the screening by IC–MS to detect possible anionic metabolites in the urine, only HEAA, DGA, and very small amounts of GA and OA were detected, whereas no other anionic metabolites were present (Besenhofer et al., 2010). Nevertheless, urine samples were not treated with deconjugating methods, such that glucuronide or sulfate conjugates of DEG metabolites might have been present and could have accounted for part of the unexplained dose.

Metabolic acidosis is a distinguishing characteristic of DEG toxicity in animals and humans (Alfred et al., 2005; Heilmair et al., 1993). Because there are several acid metabolites of DEG, these studies were conducted to evaluate which acid was the most likely mechanism for the development of metabolic acidosis. Blood HEAA concentrations were 100-fold higher than DGA, GA, or other acids, suggesting that HEAA accumulation is the most likely explanation. Also, blood HEAA levels strongly correlated with the increase in the anion gap and the decrease in blood bicarbonate. Furthermore, the increase in blood HEAA appeared to account for the decrease in blood bicarbonate (because the summed levels in the acidic animals were not statistically different from the summed levels

![Graph](https://academic.oup.com/toxsci/article-abstract/123/2/374/1690069/381)

**FIG. 6.** Blood HEAA concentrations correlate with the development of acidosis, indicated by the anion gap (left) and the blood bicarbonate concentration (right). Individual data points are plotted using the values from high-dose–treated rats only because other groups showed no acidosis.

**FIG. 7.** HEAA concentrations account for the decrease in bicarbonate concentrations in DEG-treated rats. Blood bicarbonate concentrations from the various treatment groups are plotted on the left. Blood bicarbonate + blood HEAA concentrations were added together for individual rats, then grouped. The summed values are plotted on the right. Data are represented as means ± SEM (n = 6 per group). Asterisk (*) indicates significant difference from control rats (p < 0.05); the summed values were not significantly different between high-dose DEG-treated rats and control rats.
in the nonacidic controls). The small difference in bicarbonate depletion compared with HEAA accumulation may have resulted from an accumulation of other acid metabolites of DEG or from accumulation of lactate due to a metabolic effect (Heilmair et al., 1993), such as occurs in late stage methanol poisoning (Jacobsen and McMartin, 1986). One caveat of the present study is that blood lactate levels were not measured. Another possible explanation for the observed metabolic acidosis might be contributions from the liver injury or renal failure; however, the acidosis peaked at 8–12 h and was diminishing by the time that kidney failure was evident (36–48 h).

In human case studies, the lethal dose of DEG has been estimated to be about 1–1.5 g/kg body weight (Calvery and Klumpp, 1939; O’Brien et al., 1998; Wax, 1995), which is significantly lower than the 10 g/kg dose that produced toxicity in our studies. Factors that could contribute to this discrepancy include an underestimation of the toxic dose in humans because of the frequently unreliable nature of ingestion histories by patients or family members, an increased sensitivity to DEG in humans compared with rats, or a difference in the regimen by which DEG was ingested/dosed. Typical epidemic DEG poisonings occur when humans are repeatedly exposed to a contaminated medication over several days (Ferrari and Giannuzzi, 2005), but in our study, only a single large dose was administered. It is possible that repeated ingestion of small doses of DEG by rats (similar to the toxic dose in humans) could produce similar effects. Given the apparent concentrative ability of kidney and liver tissues to retain DGA, multiple small doses of DEG could likely lead to toxic levels of metabolites in the target organs by similar mechanisms in both humans and rats.

In conclusion, these studies have shown for the first time that DGA was greatly accumulated by the kidney following DEG treatment, such that tissue concentrations of DGA were about the same as those of HEAA by the time that kidney toxicity was evident. Tissue levels of the two metabolites were increased only at high toxic doses of DEG, and the kidney levels of both metabolites correlated well with the degree of renal damage. HEAA was found in much higher concentrations in the blood than was DGA and so HEAA is the likely metabolite responsible for the production of metabolic acidosis. Although the surprisingly high level of renal accumulation of DGA portends a potential role in the kidney toxicity, in vitro studies will be needed to delineate whether DGA or HEAA is the likely cause of the renal toxicity of DEG. Hence, these studies have presented a challenge for future studies, to differentiate the renal effects of DGA from that of HEAA and to assess their respective roles in producing the renal damage elicited by DEG.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

FUNDING

Ethylene Glycol/Ethylene Oxide Panel of the American Chemistry Council. Members of the panel include BASF Corporation, Dow Chemical Company, Eastman Chemical Company, and Shell Chemical.

ACKNOWLEDGMENTS

The authors would like to thank Dr Nigel Moore for the critical review of this manuscript. K.E.M. receives royalties from Mericon Investment Group through the sales of Antizol, one of several products containing fomepizole, for the treatment of methanol or EG poisoning; fomepizole is not approved for marketing for the treatment of DEG poisoning. There is no interest to disclose for this manuscript for any of the other authors.

REFERENCES


