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Characterizing concentrations of diethylene glycol and suspected metabolites in human serum, urine, and cerebrospinal fluid samples from the Panama DEG mass poisoning

J. G. SCHIER^{1,2}, D. R. HUNT¹, A. PERALA³, K. E. MCMARTIN⁴, M. J. BARTELS³, L. S. LEWIS¹, M. A. MCGEEHIN¹, and W. D. FLANDERS¹

¹Division of Environmental Hazards and Health Effects, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

²Department of Emergency Medicine, Emory University School of Medicine, Atlanta, GA, USA

³The Dow Chemical Company, Atlanta, GA, USA

⁴LSU Health Sciences Center – Shreveport, Shreveport, LA, USA

Abstract

Context—Diethylene glycol (DEG) mass poisoning is a persistent public health problem.

Unfortunately, there are no human biological data on DEG and its suspected metabolites in poisoning. If present and associated with poisoning, the evidence for use of traditional therapies such as fomepizole and/or hemodialysis would be much stronger.

Objective—To characterize DEG and its metabolites in stored serum, urine, and cerebrospinal fluid (CSF) specimens obtained from human DEG poisoning victims enrolled in a 2006 case-control study.

Methods—In the 2006 study, biological samples from persons enrolled in a case-control study (42 cases with new-onset, unexplained AKI and 140 age-, sex-, and admission date-matched controls without AKI) were collected and shipped to the Centers for Disease Control and Prevention (CDC) in Atlanta for various analyses and were then frozen in storage. For this study, when sufficient volume of the original specimen remained, the following analytes were quantitatively measured in serum, urine, and CSF: DEG, 2-hydroxyethoxyacetic acid (HEAA), diglycolic acid, ethylene glycol, glycolic acid, and oxalic acid. Analytes were measured using low resolution GC/MS, descriptive statistics calculated and case results compared with controls when

Address correspondence to: Joshua G. Schier, MD, MPH, CDC/NCEH/EHHE/HSB MS F-60, 4770 Buford Hwy, Chamblee, GA 30341, USA. Tel: + 770 488 3401. jschier@cdc.gov.

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Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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appropriate. Specimens were de-identified so previously collected demographic, exposure, and health data were not available. The Wilcoxon Rank Sum test (with exact p-values) and bivariable exact logistic regression were used in SAS v9.2 for data analysis.

Results—The following samples were analyzed: serum, 20 case, and 20 controls; urine, 11 case and 22 controls; and CSF, 11 samples from 10 cases and no controls. Diglycolic acid was detected in all case serum samples (median, 40.7 mcg/mL; range, 22.6 – 75.2) and no controls, and in all case urine samples (median, 28.7 mcg/mL; range, 14 – 118.4) and only five (23%) controls (median, <Lower Limit of Quantitation (LLQ); range, <LLQ–43.3 mcg/mL). Significant differences and associations were identified between case status and the following: 1) serum oxalic acid and serum HEAA (both OR = 14.6; 95% C I = 2.8 – 100.9); 2) serum diglycolic acid and urine diglycolic acid (both OR >999; exact $p < 0.0001$); and 3) urinary glycolic acid (OR = 0.057; 95% C I = 0.001–0.55). Two CSF sample results were excluded and two from the same case were averaged, yielding eight samples from eight cases. Diglycolic acid was detected in seven (88%) of case CSF samples (median, 2.03 mcg/mL; range, <LLQ, 7.47).

Discussion—Significantly elevated HEAA (serum) and diglycolic acid (serum and urine) concentrations were identified among cases, which is consistent with animal data. Low urinary glycolic acid concentrations in cases may have been due to concurrent AKI. Although serum glycolic concentrations among cases may have initially increased, further metabolism to oxalic acid may have occurred thereby explaining the similar glycolic acid concentrations in cases and controls. The increased serum oxalic acid concentration results in cases versus controls are consistent with this hypothesis.

Conclusion—Diglycolic acid is associated with human DEG poisoning and may be a biomarker for poisoning. These findings add to animal data suggesting a possible role for traditional antidotal therapies. The detection of HEAA and diglycolic acid in the CSF of cases suggests a possible association with signs and symptoms of DEG-associated neurotoxicity. Further work characterizing the pathophysiology of DEG-associated neurotoxicity and the role of traditional toxic alcohol therapies such as fomepizole and hemodialysis is needed.

Keywords

Kidney; CNS/psychological; Metabolic; diethylene glycol; diglycolic acid; hydroxyethoxyacetic acid

Introduction

Diethylene glycol (DEG) is a clear, colorless liquid used in the production of a wide variety of commercial and industrial products.¹ It is found in trace, non-harmful amounts in other products such as some dietary supplements and cosmetics (probably as a manufacturing contaminant).^{2,3} When ingested in large amounts (1–1.5 g/kg), DEG can be a potent nephrotoxic and neurological poison.^{1,4} Unfortunately, DEG's physical and chemical properties give it similar properties to solvents safely used in drug delivery such as propylene glycol and glycerin. This fact, and its lower cost compared with solvents such as pharmaceutical grade glycerin have resulted in more than 13 medication-associated, DEG mass poisonings. These incidents were associated with more than 500 deaths and thousands of sub-lethal exposures around the world since 1937, many of which occurred in

children.^{1,4-7} Many if not all appear to be the result of substitution for diluents such as propylene glycol and glycerin.^{4,6-7} Despite the long history of medication-associated DEG poisoning, surprisingly little is known about the pathophysiology of human disease. As a result, the role of traditional antidotal therapy used in other, similar toxic alcohol poisonings (e.g., methanol and ethylene glycol) is unclear in DEG poisoning.

DEG is not hydrolyzed to two ethylene glycol molecules (animal data only) and further metabolized to known ethylene glycol metabolites, as once thought.⁸ Limited animal and in-vitro evidence supports that DEG is metabolized to 2-hydroxyethoxy acetaldehyde by alcohol dehydrogenase (ADH) and then to 2-hydroxyethoxyacetic acid (HEAA) by aldehyde dehydrogenase.^{8,9} (Figure 1) This work has also identified diglycolic acid as a DEG metabolite in animal poisoning experiments,^{9,10} identified diglycolic acid as a substantial contributor to DEG induced acute kidney injury (AKI),^{11,12} and shown that inhibition of DEG metabolism by an ADH inhibitor such as fomepizole (4-methylpyrazole) decreases kidney injury and lethality.^{8,9} Unfortunately, there is no published information identifying the presence and/or pattern of HEAA and diglycolic acid concentrations in human DEG poisoning. Such information, if available, could be used to inform decision-making regarding whether or not to administer an agent such as fomepizole and/or perform hemodialysis. A single case report exists of a person who ingested a large amount of a pure form of one of these substances (diglycolic acid). He developed acute renal failure, peripheral neuropathy, coma, and ultimately died; unfortunately, no serum or urine toxicology testing was done.¹³ The primary objective of this study was to identify and characterize DEG metabolite patterns in human serum, urine, and cerebrospinal fluid (CSF) specimens obtained from DEG poisoning victims. Our secondary objective was to compare these results to biologic specimens obtained from control-patients and describe the implications of these findings. This information may help clinicians characterize and quantify the severity and extent of human DEG poisoning by 1) identifying potential biomarkers for further validation; 2) informing laboratory assay development; 3) assisting clinicians in interpreting future biological testing results, and 4) provide data which can be used to inform future research and clinical decision-making.

Methods

Sample collection

All biological samples (serum, urine, and CSF) used in this study were obtained during an epidemiological investigation of a DEG mass poisoning in the Republic of Panama in 2006. This outbreak resulted when diethylene glycol was substituted for an appropriate diluent and used to formulate a sugarless cough syrup.⁷ Biological samples from persons enrolled in a case-control study (42 cases with new-onset AKI and 140 age-, sex-, and admission date-matched controls without AKI) were collected and shipped to the Centers for Disease Control and Prevention (CDC) in Atlanta for various analyses. Confirmatory laboratory testing for blood or urine DEG concentrations were not readily available during the actual outbreak and therefore could not be used in identifying cases for the case-control study. However, later testing by CDC on a sub-sample of urine specimens from the study revealed significantly higher urinary DEG concentrations in cases compared with controls.⁷ Results

of the case-control study overwhelmingly implicated a locally produced cough syrup that was formulated with DEG. More detailed data on cases and controls are published (demographic, comorbidities, etc...) ⁷; however, descriptive information on actual exposure time and duration is limited. In the original outbreak, most exposures occurred one or more times daily, over several days. The samples analyzed for this project were collected during the 2006 case-control study. They were in general, collected several days after exposure began and at varying intervals for each case. ⁷ Appropriate public health measures were taken in response to these findings at the time. ⁷ The Panamanian biological samples were then stored at -70°C until internal funding to support this project became available. For this study, we analyzed biological samples that had sufficient volume remaining for the analysis from the original samples obtained from the 42 cases and 140 controls back in 2006. These included 20 case serum (48%) and 11 case urine (28%) specimens along with 20 control serum (14%) and 22 control urine (16%) specimens. Eleven case CSF samples representing 10 patients that had neurological signs and symptoms were identified among the stored samples and analyzed. No control patient CSF samples were available for analysis. A single CSF specimen made from pooled CSF from 30 to 40 donors originally used for the matrix blank was also tested for all analytes.

Laboratory analysis

Samples were shipped frozen, on dry ice from the CDC to Dow Chemical Company for analysis due to their previous work analyzing animal biologic specimens for DEG and its metabolites. ⁹ The following analytes were measured in serum, urine, and CSF: DEG, ethylene glycol, glycolic acid, oxalic acid, HEAA, and diglycolic acid. Diglycolic acid and HEAA were chosen because they were either detected or suspected as possible metabolites in animal models of DEG poisoning. ⁸ Concentrations of ethylene glycol and its metabolites (glycolic and oxalic acid) were studied because of the unlikely, but theoretical, possibility of DEG metabolism to ethylene glycol in humans. These analytes were primarily chosen because of previous work that had resulted in analytical methods already developed to detect them in rat studies, which could be readily adapted to human samples. ⁹ The analyte 1,4-dioxanone, a hypothesized molecule that may originate from HEAA only when analytical preparations involve strong acidification, was not included for this reason. ⁸

Sample analysis was conducted using chemical derivatization with gas chromatography (GC) using electron impact ionization or methane negative chemical ionization (NCI) with mass spectrometry (MS) detection (GC/EI/MS or GC/NCI/MS) for DEG and ethylene glycol and the four acid metabolites (glycolic acid, oxalic acid, HEAA, and diglycolic acid), respectively. The GC/MS methods were modifications of our previously reported techniques for ethylene glycol, glycolic acid, and oxalic acid. ¹⁴ Stable isotope-labeled analogs of each analyte were employed as quantitative internal standards in the assays. All analyses were performed on a Phenomenex (Torrance, CA, USA) ZB-5ms column (30 m X 0.25 mm I.D. \times 0.50 μm film). Helium carrier gas was used in constant flow mode. The following ions were used for quantitation with the MS operated in the SIM mode: GA, m/z 247.1; 13C-GA, m/z 248.2; HEAA, m/z 291.2; D6-HEAA, m/z 297.2, OA, m/z 261.1; 13C2-OA, m/z 263.1, DGA, m/z 305.2; D4-DGA, m/z 309.2 with dwell time of 100 ms for all ions and internal standards. Additional confirmation ions were not incorporated into the assay, due to the lack

of significant, compound-specific fragment ions and based on the extremely low concentrations of metabolites (EG, GA, OA, HEAA, and DGA) present in human samples. The use of stable-isotope labeled internal standards, however, confirmed analytes' retention times for each sample analyzed. Also, the retention time and mass spectral response for each analyte was as a chemical derivative, providing further specificity to these analyses.

All matrices purchased for preparation of matrix standards were pooled from male and female donors who gave informed consent and were de-identified. Sample preparation for DEG and ethylene glycol by GC/MS in human samples was performed by adding urine, serum, or CSF sample aliquots to Milli-Q water. An aliquot of internal standard solution was added to each sample, followed by 5 N NaOH, toluene, and pentafluorobenzoyl chloride. The sample was vortex-mixed and heated 50°C for 1 h, and then centrifuged at 3400 rpm for 10 min. The toluene layer was removed and analyzed by GC/NCI/MS.

Sample preparation for acid metabolites by GC/MS in human samples was performed by adding serum samples to acetonitrile at a 1:1 ratio in a centrifuge tube to precipitate proteins; the sample was briefly vortex-mixed followed by centrifugation at 15000 rcf for 10 min. An aliquot of urine, CSF or serum supernatant + 1 N HCl, was added to glass vial and capped. Internal standard solution was added followed by methyl-tert butyl ether (MTBE) containing 0.5% trioctylphosphine oxide. Each sample was vortex-mixed for 30 min and the MTBE layer was transferred to a clean 2-mL glass auto sampler vial, the extraction step was repeated and the extracts were combined. Samples were blown to dryness under a nitrogen stream and then reconstituted in toluene and *N*-(*tert*-Butyldimethylsilyl)-*N*-methyltrifluoroacetamide derivatization reagent was added. Each vial was capped with a Teflon-lined crimp cap, heated at 60°C for 1 h, and then analyzed by GC/EI/MS.

Data analysis

Detection frequencies and descriptive statistics (median and range) were calculated. For observations in which the quantitative result was less than the lower limit of quantitation (LLQ), the LLQ divided by the square root of two was used instead in order to determine median concentration (serum, urine, and CSF). This was only done to determine median concentration for analytes that had more than 50% of their values above the LLQ. Analyte concentrations less than the LLQ were considered non-detectable and results analyzed by exact bivariable logistic regression (SAS v9.2) to determine the association of detection with case status. Analytes not significantly associated with case status by this method, but which had detectable concentrations in at least 90% of cases and 90% of controls, were dichotomized at the median and re-analyzed by exact logistic regression. The Wilcoxon Rank Sum test (with exact p-values) was also used to analyze the serum and urine results.

Simple quantitative descriptive statistics (median, range) were used to characterize CSF analyte concentrations. Two CSF samples were from a single subject; those values were averaged to produce a single result for analysis. Two samples were excluded from the analysis because we could not confirm that one originated from a case patient and one was from a case excluded from the original study. This left eight values, representing eight patients. All samples in the analysis originated from cases with neurological findings (e.g., flaccid paralysis, extremity weakness, facial palsy, etc.) previously associated with DEG

poisoning.¹⁵ For comparison purposes, analyte testing results on the single CSF sample used as a matrix blank, which consisted of CSF from a pool of 30–40 persons obtained from a commercial bio-bank, is presented.

Although descriptive data were available for the 2006 study participants, informed consent for this investigation could not be obtained for a variety of reasons including subject loss to follow-up, deaths and difficulty in contacting patients from another country. Therefore all biological specimens were de-identified. The CDC Institutional Review Board determined this protocol to be exempt from review. The Dow Chemical Company's Human Subjects Research Board approved the protocol.

Results

Twenty serum specimens from both cases and controls were analyzed. Eleven and 22 urine specimens were analyzed from cases and controls, respectively. Urinary oxalic acid concentration could not be determined because of strong matrix effects encountered during laboratory analysis. One case had insufficient quantities of urine to perform testing for glycolic acid, HEAA, and diglycolic acid and was excluded from the analysis.

Diglycolic acid was detected in all case serum and urine samples. Diglycolic acid was not detected in any control serum samples but was detected in five (22.7%) control urine samples (Table 1). The median case serum diglycolic acid concentration was 40.7 mcg/mL and the median case urine diglycolic acid concentration was 28.7 mcg/mL. In controls, the median value for each was less than the lower limit of quantitation. The median and ranges for all analytes in serum and urine in cases and controls are presented in Table 2.

When considered dichotomously (detected or non-detected), the presence of diglycolic acid was the only analyte significantly associated with case status (serum and urine, OR=>999; exact $p < 0.0001$) (Table 1). Some analytes (serum oxalic acid, glycolic acid, and HEAA along with urinary glycolic acid) had sufficient numbers (>90%) of samples with quantifiable concentrations in both cases and controls to analyze by exact logistic regression when dichotomized at the median into a "high" and "low" group. This demonstrated that elevated serum oxalic acid and serum HEAA concentrations were also significantly associated with case status (OR = 14.6; 95% CI= [2.8,101], for both analytes). Urinary glycolic acid concentrations were significantly lower among cases when compared with controls (OR=0.057; 95% CI=[0.001,0.546]). The Wilcoxon Rank Sum test gave similar results to the above-mentioned logistic regression analyses for serum (oxalic acid, HEAA, and diglycolic acid being higher in cases vs. controls) and urine (glycolic acid and diglycolic acid being lower and higher in cases vs. controls, respectively) (exact $p < 0.0001$).

For the CSF samples ($n=8$), detection frequencies were as follows: DEG ($n= 3$; 38%), ethylene glycol ($n=0$), glycolic acid ($n=8$; 100%), HEAA ($n=5$; 63%), and diglycolic acid ($n= 7$; 88%). Median concentrations and ranges for all analytes in mcg/mL were as follows: DEG (<LLQ; range, <LLQ–4.82), glycolic acid (2.84; range, 1.77–3.77), HEAA (1.01; range, <LLQ–121), and diglycolic acid (2.03; range, < LLQ–7.47). The sole CSF specimen

used to develop the matrix blank had non-detectable concentrations for each analyte except for glycolic acid (2.91 µg/mL).

Discussion

Diglycolic acid and HEAA were identified in human DEG poisoning among not just one biological matrix, but three (serum, urine, and CSF). The strong association of diglycolic acid (serum and urine) and HEAA (serum only) with case status strongly suggests DEG as the etiology. The most striking results are the marked differences in serum and urine diglycolic acid concentration between cases and controls. It is interesting to briefly compare these results to those reported in DEG poisoned rats by oral gavage. The median diglycolic acid serum concentration of 41 µg/mL is approximately 0.3 mmol/L, which is much higher than concentrations reported in high-dose (10 g/kg) exposed rats (mean, 0.04 mmol/L; range, <LLQ, 0.2 mmol/L).¹⁰ This might be due to a different exposure scenario from the single, acute ingestion model used in these rat studies. In the original Panama mass poisoning, patients had been instructed to consume a dose of the implicated cough syrup containing DEG as much as three times a day for as long as needed. The kinetic data in rats also showed that diglycolic acid concentrations peaked at a later time than HEAA in serum. This might explain the high serum and urine diglycolic acid concentrations seen in our human cases relative to other analytes, since biological samples from cases were collected well after onset of illness.⁷ Other animal and in-vitro work in cultured human kidney cells implicate diglycolic acid as a nephrotoxic agent in DEG poisoning.^{11,12} The in-vitro work suggests that HEAA is not nephrotoxic.¹¹ Diglycolic acid appears to be the most likely etiology of DEG's nephrotoxicity.^{11,12}

A previous study did not detect urinary diglycolic acid in a rat model of DEG poisoning.⁸ The authors hypothesized that HEAA might have formed a cyclic compound, 1,4-dioxanone, not subject to further degradation. However, they used DEG doses of 1.1 g/kg which may have been too small to produce substantial diglycolic acid concentrations.⁸ Similar rat models have found detectable urinary diglycolic acid concentrations following higher DEG doses, from 2 to 10 g/kg.¹⁰ Furthermore, the analytical methods used by Weiner et al. (high-performance liquid chromatography)⁸ may have been unable to detect very small amounts of diglycolic acid, even if present, as compared with the GC/MS method used in later work.¹⁰ Rat studies with dioxane suggest that HEAA and 1,4-dioxanone may co-exist in a pH-dependent equilibrium that is heavily favored toward HEAA,¹⁶ although 1,4-dioxanone may be favored in very acidic environments (<pH 5).¹⁶⁻¹⁸ However, such a pH is not encountered even in the urine of rats with severe acidosis from high doses of DEG⁹ and this compound is not observed under normal aqueous conditions in vivo.¹⁶

Case serum and urine DEG concentrations were not significantly higher than controls. This is not surprising given that biological samples were typically collected days after last exposure to the cough syrup, allowing sufficient time for DEG metabolism (half-life of 5–13 h).^{5,7,10} The few control samples that did have detectable serum DEG concentrations may have resulted from background environmental exposures as DEG can be found in packaging materials, cosmetics, and certain foodstuffs such as dietary supplements.^{2,3} During the initial outbreak investigation in 2006, urinary DEG concentrations were determined by CDC

on a small sample of case and control-patient specimens: a significant difference was found.⁷ The reason for the difference in findings between the 2006 study and this one may be due to different samples being tested although the possibility that DEG underwent some degradation while in storage is possible.

Case serum samples had significantly higher HEAA concentrations compared with controls as expected given that HEAA is a known metabolite of DEG. A possible explanation for the fact that many control samples had low but detectable serum HEAA concentrations is background environmental exposure. This may have occurred from personal care products containing either DEG as noted above or 1,4-dioxane which is metabolized to HEAA in vivo.^{19,20} Three samples (one case serum, one case urine, and one control urine sample) contained detectable ethylene glycol concentrations. This may be due to background environmental exposure among controls or possibly from further reaction of one of the DEG acid or aldehyde intermediates¹⁰ (not by DEG metabolism to two ethylene glycol molecules). Finally, although control patients in the study had to have been hospitalized for any condition besides AKI,⁷ they could have received the implicated DEG-containing cough syrup while in the hospital. The implicated medication was formulated in a pharmaceutical manufacturing plant operated by the hospital system and distributed to patients of this system. This might also explain why a few control-patients had detectable concentrations of DEG and HEAA.

Urinary glycolic acid concentrations were significantly lower among case samples when compared with controls. Limited human data suggest that normal urinary glycolic acid concentrations are approximately 38.8 mg/day (SD: 13.8 mg) and depend largely on diet.²¹ If one assumes an average adult urine volume of 1.5 L, control patients were probably excreting similar amounts, approximately 22.5 mg/day (15 mcg/mL × 1500 mL/day) of glycolate. These values are proportionally much greater than the median urinary glycolic acid concentrations seen among cases (3 mcg/mL × 1500 mL/day or 4.5 mg/day). The lower values among cases may be due to an impaired ability to excrete glycolic acid due to AKI from DEG's nephrotoxic effects. Impaired kidney function in cases may have reduced elimination of glycolic acid in urine, resulting in the availability of serum glycolic acid for further metabolism. As glycolic acid was present in the serum due to AKI, it may have been metabolized to oxalic acid (as well as other unmeasured organic acids) via normal metabolic pathways²² and oxalate accumulated in the serum of the cases due to impaired kidney function. Additionally, late sample collection relative to exposure may have resulted in simply missing the peak serum glycolic acid concentration. Unfortunately, our samples did not capture serial measurements over time and alternative explanations for these findings may exist. As expected, almost all cases and controls did not have detectable serum and urine ethylene glycol concentrations.

The pathophysiology of DEG-induced neurotoxicity is much less clear and unstudied. The detection of HEAA and diglycolic acid in nearly all case CSF samples suggests that these metabolites may be associated with DEG-associated neurotoxicity. Signs and symptoms of non-inebriation related neurotoxicity tend to only appear following nephrotoxicity,¹ hence these findings make intuitive sense (the nephrotoxic agents seem likely to also be the neurotoxic agents). Unfortunately, the data and the study design were insufficient to

adequately characterize this issue and make a determination on causation. True control CSF samples with undetectable concentrations of DEG metabolites would have enabled us to say more; alas none were available for testing. Nevertheless, the single bio-bank supplied CSF sample had no detectable HEAA or diglycolic acid concentrations providing at least one comparison value. The finding of glycolic acid in the bio-bank CSF sample is not unexpected since it is an endogenous metabolic intermediate that is likely present at some small level in all body fluids.

Taken together, these findings suggest that the DEG metabolites diglycolic acid and HEAA are associated with human DEG poisoning: these results appear similar to those found in animal models of DEG poisoning. Although this study did not assess causation, these data provide further insight into the metabolism of DEG in humans after poisoning and more data to inform future work in studying potential DEG poisoning therapies. Although the role of HEAA and diglycolic acid in DEG-associated neurotoxicity remains unclear, the limited CSF findings from this study suggest a possible association. The possibility that other unmeasured analytes may occur following DEG poisoning exists as well and these may also contribute to poisoning.

Limitations

The initial outbreak study was a case-control study that employed a matched study design. Due to the need for subject de-identification, we could not perform a matched, multivariable analysis. The true association between diglycolic acid and case status may be stronger than what we found, since ignoring the matching in the analysis of matched data typically biases results toward the null.²³ Another limitation is that the time interval between last dose and biological sample collection is unknown for each case. This probably resulted in substantial variation between analyte concentrations among cases, which limits the utility in using the quantitative data to examine dose-response relationships. We also did not measure 1,4-dioxanone concentrations due to the unlikelihood of its presence at the urine and blood pH that are encountered in human DEG poisoning; such measurements would have further supported that this compound is not formed in appreciable amounts in vivo (consistent with rat studies).¹⁶ Nor did we measure dioxane which can be metabolized to HEAA and might have affected HEAA measurements and their interpretation. It is likely that many of the cases were receiving hemodialysis during their clinical course. This may have affected analyte concentrations and quantitative measurements should be interpreted with this possibility in mind. Finally, the quantitative methods used for the laboratory analysis lacked the necessary specificity to exclude with complete certainty all other substances that have the same retention time and mass spectra on the low resolution GC/MS methods used. Further refinement of these methods to include detection of two confirmatory ions, in addition to the primary ion of interest, is needed to completely eliminate any other possibility. Nevertheless, we believe the likelihood of an alternative substance mimicking any of these analytes on GC/MS is extremely low based on several factors. The use of stable-isotope labeled internal standards confirmed analytes' retention times for each sample analyzed. Limited analyses of the CSF samples by a complementary ion chromatography–mass spectrometry method, used on previous animal studies with DEG, afforded comparable results to the GC/MS data presented here (data not shown). Additionally, most of the

measured metabolite concentrations are consistent with previously published animal DEG pharmacokinetic data.

Conclusion

Diglycolic acid (serum and urine) and HEAA (serum) concentrations are identifiable in, and associated with, human DEG poisoning. Diglycolic acid and perhaps HEAA appear to be useful biomarkers for human DEG poisoning but require further validation. These results may also be useful in interpreting biological testing results in future instances of DEG-associated illness. Further work characterizing the role of ADH inhibiting therapies such as fomepizole and ethanol, as well as hemodialysis, in treating DEG poisoning is needed. Finally, more work in human specimens is needed to validate these findings and to elucidate the role of HEAA and diglycolic acid in DEG-associated neurotoxicity.

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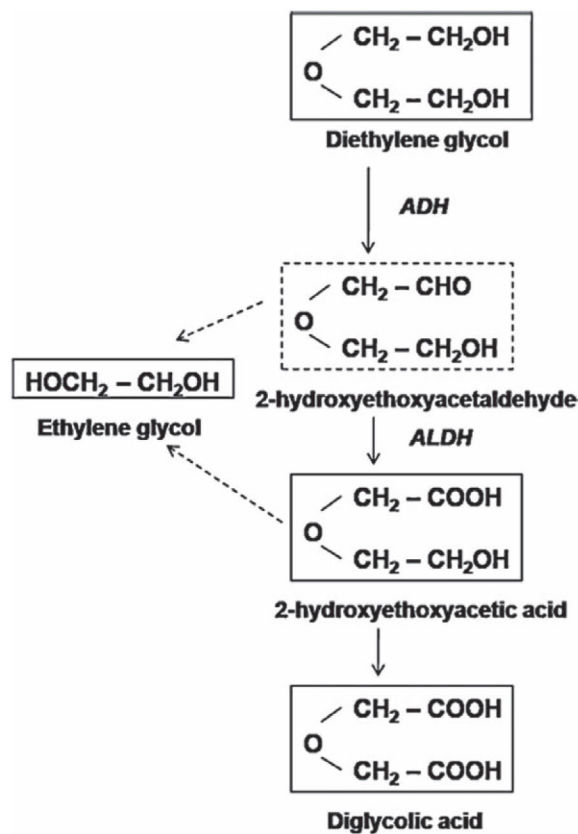


Figure 1.

Metabolic pathway for DEG based on previous animal studies and on the results presented in this report. Metabolites in lined boxes have been observed following administration of DEG to animals; those in dashed boxes are theoretical intermediates. Because fomepizole reduced the amount of EG in the urine, its origin is shown as coming from the aldehyde or acid intermediate, rather than from DEG itself. ALDH, aldehyde dehydrogenase. DGA is also known as oxybisacetic acid. Reprinted with permission from Reference # 9: Besenhofer LM, Adegboyega PA, Bartels M, et al. Inhibition of metabolism of DEG prevents target organ toxicity in rats. *Toxicol Sci* 2010;117(1):25–35.

Detection frequencies of DEG, ethylene glycol, and their toxic metabolites among stored serum and urine samples collected from participants enrolled in a case-control investigation into an outbreak of DEG poisoning in the Republic of Panama – 2006.

Table 1

Analyte	Serum		Urine*		CSF**	
	Cases (n=20)	Controls (n=20)	Cases (n=11)	Controls (n=22)	Cases (n=8)	Non-cases (n=1)
DEG	11/20 (55%)	6/20 (30%)	1/11 (9%)	2/22 (9%)	3/8 (38%)	0/1
Ethylene glycol	1/20 (5%)	0/20	1/11 (9%)	1/22 (4.6%)	0/8	0/1
Glycolic acid	20/20 (100%)	20/20 (100%)	10/10 (100%)	22/22 (100%)	8/8 (100%)	1/1 (100%)
Oxalic acid	20/20 (100%)	20/20 (100%)	***	***	***	***
HEAA	20/20 (100%)	18/20 (90%)	1/10 (10%)	1/22 (4.6%)	5/8 (63%)	0/1
Diglycolic acid †	20/20 (100%)	0/20	10/10 (100%)	5/22 (22.7%)	7/8 (88%)	0/1

* There was insufficient urine volume from one subject to determine oxalic acid, HEAA, and diglycolic acid concentrations, hence there were only 10 cases with determined analyte concentrations.

** CSF samples were from cases with neurological signs and symptoms of DEG poisoning. No control-patient specimens were available but a single CSF specimen used during the laboratory analysis as the matrix blank (pooled specimen from 30 to 40 donors) was analyzed and is presented for comparison purposes.

*** Urinary and CSF oxalic acid not able to be measured due to strong matrix effects encountered during analysis.

† In serum and urine samples, when analyte concentration was considered as a dichotomous variable (detected or non-detected) diglycolic acid was the only analyte significantly associated with case status when compared with controls (OR>999, exact $p<0.0001$).

Median concentration and ranges for diethylene glycol, ethylene glycol, and their toxic metabolites in biological fluids among participants enrolled in a case-control study of an outbreak of DEG poisoning in the Republic of Panama - 2006 (all results in mcg/mL).

Table 2

Medium	DEG		Ethylene glycol		Glycolic Acid		Oxalic Acid*		HEAA		Diglycolic acid	
	Cases (n=20)	Controls (n=20)	Cases (n=20)	Controls (n=20)	Cases (n=20)	Controls (n=20)	Cases (n=20)	Controls (n=20)	Cases (n=20)	Controls (n=20)	Cases (n=20)	Controls (n=20)
Serum	0.037; [<LLQ, 0.24]	<LLQ; [<LLQ, 0.18]	<LLQ; [<LLQ, 0.22]	<LLQ; [<LLQ, <LLQ]	2.78; [2.41, 3.27]	2.74; [2.19, 4.18]	12.96 [7.58, 30.82]	5.24; [3.59, 17.71]	1.44; [0.53, 64.79]	0.702; [<LLQ, 1.97]	40.73; [22.61, 75.15]	<LLQ; [<LLQ, <LLQ]
	Cases (n=11)	Controls (n=22)	Cases (n=11)	Controls (n=22)	Cases (n=10)	Controls (n=10)	Cases (n=0)	Controls (n=0)	Cases (n=10)	Controls (n=22)	Cases (n=10)	Controls (n=22)
Urine**	<LLQ; [<LLQ, 0.67]	<LLQ; [<LLQ, 106]	<LLQ; [<LLQ, 9.59]	<LLQ; [<LLQ, 1.16]	3.00; [1.23, 82.92]	14.73; [1.92, 43.33]	<LLQ; [<LLQ, 0.39]	<LLQ; [<LLQ, 547]	<LLQ; [<LLQ, 0.39]	<LLQ; [<LLQ, 547]	28.71; [13.99, 118.39]	<LLQ; [<LLQ, 43.29]
	Cases (n=8)	Non-case (n=1)	Cases (n=8)	Non-case (n=1)	Cases (n=8)	Non-case (n=1)	Cases (n=8)	Non-case (n=1)	Cases (n=8)	Non-case (n=1)	Cases (n=8)	Non-case (n=1)
CSF***	<LLQ; [<LLQ, 4.82]	<LLQ	<LLQ	<LLQ	2.84 [1.77, 3.77]	2.91	1.01; [<LLQ, 121]	<LLQ	1.01; [<LLQ, 121]	<LLQ	2.03; [<LLQ, 7.47]	<LLQ
	Cases (n=8)	Non-case (n=1)	Cases (n=8)	Non-case (n=1)	Cases (n=8)	Non-case (n=1)	Cases (n=8)	Non-case (n=1)	Cases (n=8)	Non-case (n=1)	Cases (n=8)	Non-case (n=1)

Serum LLQ(mcg/mL): DEG, 0.020; ethylene glycol, 0.020; glycolic acid, 0.51; oxalic acid, 1.03; HEAA, 0.51; and diglycolic acid, 10.10.

Urine LLQ (mcg/mL): DEG, 0.051; ethylene glycol, 0.10; glycolic acid, 0.26; oxalic acid, N/A; HEAA, 0.26; and diglycolic acid, 1.26.

CSF LLQ (mcg/mL): DEG, 0.051; ethylene glycol, 0.10; glycolic acid, 0.51; oxalic acid, N/A; HEAA, 0.51; and diglycolic acid, 1.01.

* Urinary and CSF oxalic acid not able to be measured due to strong matrix effects encountered during analysis.

** There was insufficient urine volume to complete the glycolic acid, HEAA and diglycolic acid assays for one case and this case was excluded from the analysis.

*** No control-patient samples were available for analysis. Quantitative results for all analytes in a single CSF specimen from 30 to 40 pooled donors used for the laboratory matrix blank is presented for comparison.

<LLQ= less than the lower limit of quantitation.