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Diglycolic acid is associated with diethylene glycol poisoning in humans

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Abstract

Diglycolic acid is associated with diethylene glycol poisoning in humans

By Joshua G Schier MD

In 2006, an outbreak of diethylene glycol (DEG) poisoning occurred in Panama. Biological samples from case and control patients enrolled in a study were shipped to Centers for Disease Control and Prevention (CDC) laboratories. After the outbreak was halted, samples were stored at CDC until 2009 when this study began. Our primary objective was to characterize the relationship between DEG and its toxic metabolites in biological samples collected from study participants. Serum and urine samples from cases and controls that had sufficient remaining volume were analyzed for DEG and its known toxic metabolites hydroxyethoxyacetic acid (HEAA) and diglycolic acid (DGA). Ethylene glycol (EG) and its toxic metabolites glycolic acid (GA) and oxalic acid (OA) were also measured. Analytes were measured using low resolution GC/MS. Detection frequencies and descriptive statistics were calculated. The Wilcoxon Rank Sum test (with exact p-values) and bivariable exact logistic regression were used in SAS v9.2 to analyze the data. Cerebrospinal fluid (CSF) samples from eight cases who developed neurotoxicity were also analyzed. Twenty case and 20 control serum samples along with 11 case and 22 control urine samples were analyzed. One case had insufficient urine volume to determine GA, HEAA and DGA concentrations. Notable results included detection of DGA in serum of all cases (median, 40.7 mcg/mL; range, 22.6-75.2) and no controls, and in the urine of all cases (median, 28.7 mcg/mL; range, 14-118.4) and only five (23%) controls (median, <LLQ; range, <LLQ-43.3 mcg/mL). The WRS test and logistic regression analyses demonstrated significant differences and associations between case status and: 1) serum OA and serum HEAA (both OR=14.6; 95%CI=2.8-100.9); 2) serum DGA and urine DGA (both OR>999; exact p<0.0009); and 3) urinary DGA (OR=0.057; 95%CI=0.001-0.55). Cerebrospinal fluid DGA concentrations were detected in 7 (88%) of CSF samples (median, 2.03 mcg/mL; range, <LLQ, 7.47). This is the first report in the literature characterizing serum, urine and CSF HEAA and DGA concentrations in human DEG poisoning and comparing them to control concentrations. Serum HEAA and DGA concentrations in serum and urine were significantly higher in cases versus controls and may be useful biomarkers in human DEG poisoning.

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Introduction

Diethylene glycol (DEG) is a clear, colorless liquid used in the production of a wide variety of commercial and industrial products including antifreeze, brake fluid, and heating/cooking fuels. (1) It can be found in trace, non-harmful amounts in other products such as some dietary supplements and cosmetics (probably as a manufacturing contaminant). (2,3) However, when ingested in amounts around 0.5-1.5 g/kg (the true minimum toxic dose in humans is unknown), DEG can be a potent, nephrological and neurological poison. (4) Unfortunately, its physical and chemical properties make DEG an exceptional solvent to deliver water-insoluble chemicals and drugs and it is much cheaper than other safer solvents, such as pharmaceutical grade glycerin. (1,5) These factors along with the failure of appropriate quality control mechanisms in various pharmaceutical manufacturing and distribution systems have resulted in its use as a diluent for many commonly available pharmaceuticals such as acetaminophen, probably for economic gain. (4,6) Tragically, DEG-laced diluents have resulted in at least 13 medication-associated, DEG mass poisonings (most of which have occurred in children) associated with more than 500 deaths and thousands of sub-lethal exposures across the world since 1937. (1,6,7) Despite the modern world's long history with DEG poisoning from these outbreaks, surprisingly little is known about the disease.

Diethylene glycol is among a group of compounds known as the "toxic alcohols," which include methanol and ethylene glycol (EG). Both methanol and EG produce toxicity by in-vivo metabolism to toxic metabolites. For some time, the nephrotoxicity of DEG was initially thought to occur as a result of cleavage of the ether bond linking the two EG molecules that make up DEG. The pathophysiology of EG induced acute renal failure (ARF) is well understood. It is metabolized by several enzymes including alcohol and aldehyde dehydrogenase, causing

excessive glycolic acid concentrations. This can result in a metabolic acidosis the severity of which is dose-dependent. The glycolic acid is then further oxidized to several other organic acids, including oxalic acid, which is excreted by the kidney. In excessive amounts, oxalic acid can react with urinary calcium to produce calcium oxalate monohydrate crystals. These crystals precipitate out in the renal tubules causing renal dysfunction and ARF. (8,9) Although initially appealing as a plausible explanation, this hypothesized mechanism of toxicity for DEG was ultimately disproven. (10,11) Traditional antidotal therapy for EG (and methanol) poisoning consists of either ethanol or fomepizole administration, both of which inhibit alcohol dehydrogenase activity and therefore stop production of toxic metabolites. (12)

Limited animal and in-vitro evidence support that DEG is metabolized to 2-hydroxyethoxy acetaldehyde by alcohol dehydrogenase and then to 2-hydroxyethoxyacetic acid (HEAA) by aldehyde dehydrogenase. Further oxidation to another possible metabolite, diglycolic acid, was minor in these studies and initially thought not to occur to any great extent. (10,11,13) These studies established that at least one or both of these metabolites (HEAA and/or diglycolic acid) is nephrotoxic in animals and inhibition of DEG metabolism by fomepizole is protective. (11) Further work demonstrated (1) substantial uptake of diglycolic acid by rodent kidney tissue in amounts 100 times that of peak serum concentrations; (2) that diglycolic acid is a major contributor to DEG induced ARF; and (3) minor amounts of EG may be produced by DEG metabolite intermediates (rather than by cleavage of the ether bond joining the two EG molecules). (13,14,15) Other than a single case report of a person who ingested diglycolic acid and developed ARF, peripheral neuropathy, coma and who ultimately died (no serum or urine testing was done), there is no published information in the medical literature on HEAA and diglyolic acid concentrations in any biological medium in humans with DEG poisoning. (16)

The extent to which any of these nephrotoxic and potentially neurotoxic metabolites occurs in human DEG poisoning is unknown: we present the first data describing DEG metabolites in human DEG poisoning and describe the implications of these findings.

The primary objective of this study was to determine if diglycolic acid production occurs in human DEG poisoning. Our secondary objectives were to 1) characterize the relationship among EG, DEG and their toxic metabolites (glycolic acid, oxalic acid, HEAA and diglycolic acid) in human DEG poisoning; and 2) characterize all of these analytes in cerebrospinal fluid (CSF) specimens obtained from a sample of cases with signs and symptoms of neurotoxicity.

Methods

Sample collection and origin

All biological samples (serum, urine, and CSF) used in this study were obtained during an epidemiological investigation of an outbreak of nephrological and neurological illness that occurred in the Republic of Panama in 2006. (7) During that outbreak, serum and urine samples of persons enrolled in a case-control study were collected and shipped to the Centers for Disease Control and Prevention (CDC) in Atlanta for various analyses. Results of the case-control study overwhelmingly implicated a locally produced cough syrup that had been formulated with DEG. (6,7) After discovery of the etiology, remaining portions of biological samples shipped to CDC from Panama during the outbreak investigation were kept at -70°C. At CDC, biological samples from public health investigations are often kept until it is determined that they are no longer needed at which point they are discarded.

For the first two objectives of this study, a case of human DEG poisoning was defined as any case enrolled in the original case-control study that had sufficient blood and urine samples remaining for analysis. (7) The original case-control study required cases to be persons admitted to a Panamanian hospital on or after August 15, 2006 who had: ARF of unknown etiology characterized by oliguria or anuria and a serum creatinine concentration $\geq$ 2 mg/dl, or who manifested an acute exacerbation of their pre-existing chronic renal failure with no other likely etiology. Controls were defined as any control patient from the original case-control study that had sufficient blood and urine samples remaining for analysis. The original case-control study required controls to be any person randomly selected from a daily census of the same hospital and matched to cases on sex, age (+/- 5 years) and admission date (within 2 days of case admission or any time thereafter) for any cause other than renal failure. In the original study there were 42 cases and 140 controls. (7) Sufficient volumes of serum and urine remained from almost half (n=20; 48%) and more than a quarter (n=11; 28%) of cases, respectively. Sufficient volumes of serum and urine for the analysis remained from 20 (14%) and 22 (16%) of controls, respectively.

In addition to the serum and urine samples collected during the case-control study, CSF samples from 8 cases with signs and symptoms of neurotoxicity were shipped to Atlanta for possible analyses.

Laboratory analysis

All analytes were measured using GC/MS via modifications of previously published methods. Sample analysis was conducted using chemical derivatization with gas chromatography

using electron impact (EI) ionization or methane negative chemical ionization (NCI) with MS detection (GC/EI/MS or GC/NCI/MS) for DEG and EG and the four acidic metabolites (glycolic acid, oxalic acid, HEAA, and diglycolic acid), respectively. The GC/MS methods were modifications of our previously reported techniques for EG, glycolic acid and oxalic acid. (20) Stable isotope-labeled analogs of each analyte were employed as quantitative internal standards in the assays. All control matrices were pooled from male and female donors, with all donors giving informed consent and personal details of donors removed from samples prior to shipment to our laboratory.

Sample preparation for DEG and EG by GC/MS in human samples was performed by added varying amounts of urine (0.5 mL), serum (0.25 mL) or CSF samples (0.25 mL) to 1.0 mL of Milli-Q water in 4-mL glass vial. A 10- µL aliquot of aqueous internal standard solution (100 µg/mL D_8-DEG and 220 µg/mL D_6-EG) was added to each sample, followed by 100 µL of 5 N NaOH, 500 µL of toluene and 50 µL of pentafluorobenzoyl chloride. The glass vial was capped with a Teflon-lined cap, vortex-mixed at 50 °C for 1 hour and then centrifuged at 3400 rpm for 10 minutes. Approximately 200 µL of 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 (0.2 mL) 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 minutes. A 200-µL aliquot of urine or serum supernatant +1.0 mL of 1 N HCl , or a 100-µL aliquot of CSF + 0.9 mL 1 N HCl + was added to 4-mL glass vial and capped with a Teflon-lined cap. Then 25 µL of the internal standard solution (~ 500 µg/mL each of ^{13}C-Glycolic Acid, $^{13}C_2$-Oxalic acid, D_4-Diglycolic acid and D_6-HEAA) was added followed by 1.0 mL of methyl-tert

butyl ether (MTBE) containing 0.5% trioctylphosphine oxide. The sample was vortex-mixed for 30 minutes and the MTBE layer was transferred to a clean 2-mL glass auto sampler vial, the extraction step was repeated and the extracts combined. Samples were blown to dryness under a nitrogen steam and then reconstituted in 450 μL of toluene and 50 μL of N-(*tert*.-Butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) derivatization reagent was added. The vial was capped with a Teflon-lined crimp cap and heated at 60 °C for 1 hour and then analyzed by GC/EI/MS.

Analyses

Detection frequencies, descriptive statistics (median and range), were calculated and the Wilcoxon Rank Sum test (with exact p-values) used in SAS v9.2 to analyze the serum and urine results. Analyte concentrations less than the lower limit of quantitation (LLQ) were considered non-detects and then all results were analyzed by exact bivariable logistic regression to determine association with case status. Analytes that were not significantly associated with case status by this method, but which had detectable concentrations in at least 90% cases and 90% of controls, were dichotomized at the median and analyzed by exact logistic regression.

Simple quantitative descriptive statistics (median, range) were used to describe the results of DEG, EG and their toxic metabolite concentrations in CSF samples. One subject had two CSF samples tested, which were averaged to produce a single result. Analyte testing results performed 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 for comparison. No control patient

CSF samples were available for analysis. Neurological signs and symptoms of the cases from which the CSF samples originated are described.

Human subjects protection

Although demographic and exposure data was available for serum and urine specimens collected during the original case-control study in 2006, informed consent from patients could not be obtained for a variety of reasons including death of many of the subjects, geography and logistical considerations (surviving patients were not able to be located). Therefore all biological specimens, including the CSF samples from participants not enrolled in the case-control study were de-identified, preventing our ability to conduct a matched analysis or take into consideration other variables. The CDC Institutional Review Board determined this protocol to be exempt from review since data was de-identified. The Dow Chemical Company's Human Subjects Research Board approved the protocol.

Results:

Case-control study

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.

The most striking finding is the preponderance of detectable diglycolic acid concentrations in both serum and urine of cases and the complete absence (serum) and near-

absence in controls. Serum samples of all cases (n=20; 100%) had detectable diglycolic acid concentrations compared with none from control patients. A similar pattern is seen again for urinary testing results where all tested cases had detectable diglycolic acid concentrations (n=10; 100%) but only a small number of samples from controls had detectable concentrations (n=5; 23%). (Table 1) Median and ranges for all analytes in serum and urine in cases and controls (except for oxalic acid in urine) are presented in Table 2.

Serum oxalic acid, HEAA and diglycolic acid concentrations were higher among cases (Wilcoxon Rank Sum test, exact p <0.0001) when compared to controls. Urinary glycolic acid concentrations were significantly lower among cases when compared to controls (exact p <0.0001). Urinary diglycolic acid concentrations were significantly higher among cases (exact p<0.0001). (Table 3)

Initially, diglycolic acid was the only analyte significantly associated with case status when compared to controls (OR >999; exact p<0.0001) in serum and urine. For the remaining analytes, sufficient numbers (at least 90%) of detectable concentrations in both cases and controls existed for serum DEG, serum EG and urinary DEG, EG, and HEAA to analyze by exact logistic regression when dichotomized at the median. Serum oxalic acid and serum HEAA were 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 to controls (OR=0.057; 95% CI=[0.001,0.546]).

Cerebrospinal fluid samples

For the CSF samples (n=8), detection frequencies were: DEG (n=3; 38%), EG (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: DEG (<LLQ, <LLQ-4.82), glycolic acid (2.84; range, 1.77-3.77), HEAA (1.01; range, <LLQ–121) and diglycolic acid (2.03 mcg/mL; 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). Neurological signs and symptoms of these cases are listed in Table 4.

Discussion

This project has for the first time in the literature, included analyses of DEG poisoning in not just one medium, but rather three (serum, urine and CSF). The biological samples collected from the Panama DEG mass poisoning presented us with an opportunity to not only characterize DEG metabolites in human DEG poisoning but to also compare results with controls. This has yielded the first insights into the potential importance of the DEG metabolites HEAA and diglycolic acid in human DEG poisoning. The most striking results are the obvious differences in diglycolic acid concentration between cases and controls in both serum and urine. Furthermore, diglycolic acid concentrations among cases were higher than any other analyte in both serum and urine samples, sometimes by as much as an order of magnitude.

It is interesting to briefly compare our diglycolic acid results to those reported in DEG poisoned rodents. The median diglycolic acid serum concentration of 41 μg/ml is approximately 0.3 mmol/L, which is much higher than concentrations reported in DEG poisoned rodents (mean, 0.04 mmol/L; range, <LLQ, 0.2 mmol/L). (14) The kinetic data in this study also showed that diglycolic acid concentrations peaked at a later time than HEAA in serum. This probably explains 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 work in animals and in human proximal tubule cells have strongly suggested that diglycolic acid is likely responsible for the renal damage induced by DEG. (11,13,14) When the findings of this study are considered with the existing aforementioned animal data, diglycolic acid is the most likely etiology of DEG's nephrotoxicity. Although these findings are the most interesting, there were several other results worth discussing.

Although serum DEG concentrations among cases were higher when compared to controls, the difference was not significant. This is not surprising given that biological samples from participants were typically collected days after last exposure to the cough syrup, allowing sufficient time for DEG metabolism. (7) Nevertheless, with more samples or with samples collected earlier, a significant difference might well have been found. The few control patients that did have detectable serum DEG concentrations may have been exposed to DEG from the environment. At least one study has documented detectable serum DEG concentrations likely resulting from their environment or diet. (3) It can be found in packaging materials, cosmetics and can be found in minor concentrations of certain dietary supplements. (2)

The significantly higher serum HEAA concentrations we saw among cases were expected: HEAA is also a known metabolite of DEG. Since HEAA exposure can result from environmental exposures to other agents such as 1,4 dioxane which is found in drinking water (17) and personal care products (18), we believe this is the likely reason for the smaller, but detectable concentrations in controls. Urinary glycolic acid concentrations were significantly lower among cases when compared to controls, which was an unanticipated finding. Glycolic acid is not an expected metabolite of DEG and our serum results are consistent with that. Limited human data suggests that normal urinary glycolic acid concentrations are approximately 38.8 mg/day (SD: 13.8 mg) and depend largely on diet. (19) Although daily urine output can vary

greatly, if one assumes an average adult urine volume of 1.5 liters, our control patients were probably excreting similar amounts, approximately 22.5 mg/day (15 mcg/mL x 1500 mL/day) of glycolate. These values are proportionally much greater than the median urinary glycolic acid concentrations seen among cases (3 mcg/mL x 1500 mL/day or 4.5 mg/day). We believe the most likely reason for this is simply that cases had ARF and although their kidneys were still able to produce some urine, their intrinsic ability to excrete glycolic acid absorbed from their diet was impaired. One would expect higher serum glycolic acid concentrations among cases if this was true; however, this was not observed. Since glycolic acid is metabolized to oxalic acid before excretion by the kidneys, one might expect significantly higher concentrations of serum oxalic acid among cases especially if kidney function was impaired: this was observed in our study. Therefore we believe it is plausible that impaired kidney function in cases reduced elimination of urinary glycolic acid, which resulted in higher serum glycolic acid concentrations at some point during our cases clinical course. As time passed and serum glycolic acid was metabolized to oxalic acid via normal metabolic pathways, serum oxalic acid concentrations climbed and impaired kidneys were unable to excrete it. Decreased urinary oxalic acid concentrations among cases would have helped support this hypothesis but unfortunately, we were not able to measure it. It is interesting to note that animal data suggest that small amounts of EG are produced in DEG poisoning, which likely result from DEG metabolism intermediates rather than from direct ether cleavage of the DEG molecule. (11) This may also contribute to higher serum oxalic acid concentrations through subsequent normal metabolic pathways. (8,9)

The pathophysiology of DEG-induced neurotoxicity is much less clear and unstudied. It is worth noting that in most other DEG mass poisoning events, the patients were children, the contaminated medication often an antipyretic like acetaminophen, and mortality was high after

illness onset. The Panama outbreak was unusual in that the affected population was adult and they received smaller doses of the DEG containing product over a more extended period of time when compared to other outbreaks. This less fulminant form of the disease likely led to longer survival times enabling the neurological manifestations to occur. The detection of HEAA and diglycolic acid in nearly all CSF samples of the DEG poisoning cases suggests that these metabolites may be associated with DEG-associated neurological disease. Cerebrospinal fluid samples from control patients would have enabled us to say this with at least some certainty, alas none were available. Nevertheless, the single CSF sample purchased from a biological bank (pooled from 30-40 donors) that was used for developing the methodology had no detectable HEAA or diglycolic acid concentrations, setting the stage for further work.

Limitations

All subjects enrolled in the original case-control study did not have individual, biological sample testing performed to confirm exposure at the time of the study. Nevertheless, aggregate data on urinary DEG concentrations obtained at the time of the original outbreak showed significantly higher concentrations among cases (0.05 – 4 mcg/mL) when compared to controls, in which DEG was undetectable ($p<0.001$). (7) The initial outbreak study was a case-control study that employed a matched study design. Due to the need to de-identify subjects with their results, we could not perform a matched study analysis. Ignoring the matching in the analysis of matched data typically results a bias towards the null, suggesting that the true associations may be stronger than those we found. (20) A further limitation is that we were not able to conduct multivariable analyses taking into consideration potential confounders such as age, gender, and other variables collected during the original case-control study. Therefore, we do not know what

effect, if any, these variables might have had on our results. 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. Finally, the quantitative methods used for this study 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. Also, the retention time and mass spectral response for each analyte was as a chemical derivative, providing further specificity to these analyses. 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). Finally, most of the measured metabolite levels fit an expected pattern based on previously published animal pharmacokinetic data with DEG, and there was an overwhelming association of diglycolic acid with cases in both serum and urine.

Conclusion

This is the first report of the DEG metabolites HEAA and diglycolic acid being detected in human serum, urine and cerebrospinal fluid specimens taken from cases of DEG poisoning. Confirmation of the presence of these analytes in human cases of DEG poisoning and the strong

association of serum and urine diglycolic acid with case status suggests that HEAA and diglycolic acid are useful biomarkers for DEG poisoning.

The existing animal literature has shown that HEAA and diglycolic acid are nephrotoxic and inhibition of DEG metabolism is protective. (8,11) This study was not designed to demonstrate that these metabolites were the nephrotoxic agents in DEG poisoning. However, the significantly higher concentrations of these metabolites in cases compared to controls provides further evidence for the need to evaluate the use of alcohol dehydrogenase inhibiting therapies such as ethanol and fomepizole in DEG poisoning. Further work in humans is needed to study this, and to elucidate the role of HEAA and diglycolic acid in DEG-associated neurotoxicity.

References

1. Schep LJ, Slaughter RJ, Temple WA, Beasley DMG. Diethylene glycol poisoning. Clinical Toxicology 2009; 47:525-35.
2. Schier JG, Barr DB, Li Z, Wolkin AF, Baker SE, Lewis LS, McGeehin MA. Diethylene glycol in health products sold over-the-counter and imported from Asian countries. J Med Toxicol. 2011 Mar;7(1):33-8.
3. Brooks JB, Basta MT, Alley CC, Holler JS, el Kholy AM. Identification of diethylene glycol in sera from Egyptian children by frequency-pulsed electron-capture gas-liquid chromatography. J Chromatogr. 1984 Aug 10;309(2):269-77.
4. O'Brien KL, Selanikio JD, Hecdivert C, Placide MF, Louis M, Barr DB, Barr JR, Hospedales CJ, Lewis MJ, Schwartz B, Philen RM, St Victor S, Espindola J, Needham LL, Denerville K. Epidemic of pediatric deaths from acute renal failure caused by diethylene glycol poisoning. JAMA. 1998 Apr 15;279(15):1175-80.
5. Winek CL, Shingleton DP, Shanor SP. Ethylene and diethylene glycol toxicity. Clinical Toxicology 1978; 13:297-324.
6. Schier JG, Rubin CS, Miller D, Barr D, McGeehin MA. Medication-associated diethylene glycol mass poisoning: a review and discussion on the origin of contamination. J Public Health Policy. 2009 Jul;30(2):127-43.
7. Rentz ED, Lewis L, Mujica OJ, Barr DB, Schier JG, Weerasekera G, Kuklenyik P, McGeehin M, Osterloh J, Wamsley J, Lum W, Alleyne C, Sosa N, Motta J, Rubin C. Outbreak of acute renal failure in Panama in 2006: a case-control study. Bull World Health Organ. 2008 Oct;86(10):749-56.

8. Herbert JL, Fabre M, Auzépy P, Paillas J. Acute experimental poisoning by diethylene glycol: acid base balance and histological data in male rats. Toxicol Eur Res. 1978;1(5):2890-94.
9. Guo C, Cenac TA, Li Y, McMartin KE. Calcium oxalate, and not other metabolites, is responsible for the renal toxicity of ethylene glycol. Toxicol Lett. 2007;173;8-16.
10. Wiener HL, Richardson KE. Metabolism of diethylene glycol in male rats. Biochem Pharmacol 1989; 38:539–541.
11. Besenhofer LM, Adegboyega PA, Bartels M, Filary MJ, Perala AW, McLaren MC, McMartin KE. Inhibition of metabolism of diethylene glycol prevents target organ toxicity in rats. Toxicol Sci. 2010 Sep;117(1):25-35. Epub 2010 Jun 7.
12. Makar AB, Tephly TR. Inhibition of monkey liver dehydrogenase by 4-methylpyrazole. Biochem Med 1975; 13:334-342.
13. Landry GM, Martin S, McMartin KE. Diglycolic acid is the nephrotoxic metabolite in diethylene glycol poisoning inducing necrosis in human proximal tubule cells in vitro. Toxicol Sci. 2011 Nov;124(1):35-44. Epub 2011 Aug 18.
14. Besenhofer LM, McLaren MC, Latimer B, Bartels M, Filary MJ, Perala AW, McMartin KE. Role of tissue metabolite accumulation in the renal toxicity of diethylene glycol. Toxicol Sci. 2011 Oct;123(2):374-83. Epub 2011 Jul 29.
15. Landry G, Dunning C, McMartin K. Diglycolic acid, the nephrotoxic metabolite of diethylene glycol, produces cytotoxicity via molecular mimicry and metabolic disruption. 2012 North American Congress of Clinical Toxicology - October 2012; Abstact #3. Available at: http://clintox.org/NACCT/2012/NACCT_Platform_Abstracts_2012.pdf

16. Roscher AA, Jussek E, Noguchi T, Franklin S. Fatal Accidental diglycolic acid intoxication. Toxicol Pathol 1975 3: 3.

17. United States Environmental Protection Agency. 1,4-Dioxane. Available at: http://www.epa.gov/chemfact/dioxa-sd.pdf. Accessed on: 7/6/2012.

18. Black RE, Hurley FJ, Havery DC. Occurrence of 1,4-dioxane in cosmetic raw materials and finished cosmetic products. J AOAC Int. 2001 May-Jun;84(3):666-70.

19. Holmes RP, Goodman HO, Hart LJ, Assimos DG. Relationship of protein intake to urinary oxalate and glycolate excretion. Kidney International, Vol. 44 (1993), pp. 366—3 72.

20. Breslow NE, Day NE. Statistical methods in cancer research. Volume I - The analysis of case-control studies. IARC Sci Publ. 1980;(32):5-338.

Table 1: Detection frequencies of diethylene glycol, 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

Analyte	Serum		Urine*	
	Cases (n=20)	Controls (n=20)	Cases (n=11)	Controls (n=22)
Diethylene glycol	11/20 (55%)	6/20 (30%)	1/11 (9%)	2/22 (9%)
Ethylene glycol	1/20 (5%)	0/20	1/11 (9%)	1/22 (4.6%);
Glycolic acid	20/20 (100%)	20/20 (100%)	10/10 (100%)	22/22 (100%)
Oxalic acid	20/20 (100%)	20/20 (100%)	**	**
Hydroxyethoxyacetic acid (HEAA)	20/20 (100%)	18/20 (90%)	1/10 (10%)	1/22 (4.6%)
Diglycolic acid***	20/20 (100%)	0/20	10/10 (100%)	5/22 (22.7%)

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

**Urinary 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 to controls (OR >999; exact p<0.0001)

Table 2: Median concentration and ranges for diethylene glycol, ethylene glycol and their toxic metabolites 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)

Medium	**Diethylene glycol**		**Ethylene glycol**		**Glycolic Acid**		**Oxalic Acid***		**Hydroxyethoxyacetic Acid**		**Diglycolic acid**	
Serum	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)
	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]
Urine**	Cases (n=11)	Controls (n=22)	Cases (n=11)	Controls (n=22)	Cases (n=10)	Controls (n=22)	Cases (n=0)	Controls (n=0)	Cases (n=10)	Controls (n=22)	Cases (n=10)	Controls (n=22)
	<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]	28.71; [13.99,118.39]	<LLQ; [<LLQ, 43.29]

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

<LLQ= less than the lower limit of quantitation

Table 3: Wilcoxon Rank Sum Test (exact) results for analyte concentrations in serum (n=40) and urine (n=33) specimens collected from participants enrolled in a case-control study of an outbreak of DEG poisoning in the Republic of Panama - 2006*

Analyte	Serum					Urine				
	Cases		Controls			Cases		Controls		
	Sum of Scores	Mean Score	Sum of Scores	Mean Score	Exact P-value	Sum of Scores	Mean Score	Sum of Scores	Mean Score	Exact P-value
Diethylene glycol	469	23.5	351	17.6	0.079	186	16.9	375	17	0.84
Ethylene glycol	420	21	400	20	1.00	193	17.5	368	16.7	0.77
Glycolic acid	413	20.7	407	20.4	0.95	84	8.4	444	20.2	<0.0001
Oxalic acid	559	28.0	261	13.1	<0.0001	**	**	**	**	**
Hydroxyethoxyacetic acid	547	27.4	273	13.7	<0.0001	170.5	17.05	357.5	16.25	1.00
Diglycolic acid	610	30.5	210	10.5	<0.0001	269	26.9	259	11.8	<0.0001

*Twenty samples of serum for cases and controls were analyzed, whereas there were only 11 case urine samples and 22 control urine samples. One case did not have sufficient urine volume to analyze for glycolic acid, HEAA and diglycolic acid hence n=10 for these analytes.

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Table 4: Neurological signs and symptoms of DEG poisoned patients from which CSF samples were obtained (n=8)

- Confusion, seizures, flaccid paralysis
- Confusion, facial nerve palsy, dysarthria, hyporeflexia in lower extremities
- Decreased motor strength in lower extremities, generalized hyporeflexia
- Seizures decreased motor strength in lower extremities, generalized hyporeflexia
- Flaccid paralysis, bilateral facial nerve palsy mydriasis, generalized areflexia
- Confusion, decreased strength in lower extremities
- Seizures, flaccid paralysis, bilateral facial palsy, lower extremity areflexia
- Bilateral facial palsy, decreased strength in lower extremities, generalized hyporeflexia