Ethylene Glycol and Glycolic Acid in Postmortem Blood from Fatal Poisonings

Thomas G. Rosano[1], Thomas A. Swift[1], Christopher J. Kranick[1], and **Michael Sikirica[2]**

[1]Department of Pathology and Laboratory Medicine, Albany Medical Center, Albany, New York and
[2]Forensic Medical Services, Waterford, New York

Abstract

Ethylene glycol (EG), a relatively infrequent cause of fatal intoxication, presents an analytical challenge for forensic confirmation in postmortem toxicology. We report EG and glycolic acid (GA) quantification in postmortem blood by gas chromatography coupled with ion trap mass spectrometry (GC–MS) analysis using a modification of a previously reported clinical method. The method is linear from 50 to 4000 mg/L with a limit of detection of 25 mg/L for both EG and GA. Interassay coefficient of variation (2.1–8.6%, 4.3–6.0%) and accuracy (96–101%, 92–105%) were determined for EG and GA, respectively. EG concentration by ion trap GC–MS correlated closely (R^2 = 0.995) with EG quantified by GC–flame-ionization detection. Analysis of blood from 20 autopsies with no evidence of EG exposure did not reveal detectable EG or GA. In 12 medical examiner cases with EG poisoning as cause of death, EG concentrations ranged widely from 58 to 7790 mg/L with a mean of 1830 mg/L, and the GA concentration averaged 1360 mg/L with a narrower range of 810–1770 mg/L. EG and GA levels correlate poorly (R^2 = 0.15) in postmortem blood with discordantly low EG concentrations in two cases. Birefringent oxylate crystals in renal tissue was a consistent finding. In conclusion, a sensitive and specific GC–MS method for detection and quantification of EG and GA has been validated and a study of fatal EG poisonings revealed forensic application of the method.

Introduction

Poisoning from ethylene glycol (EG), an odorless dihydric alcohol, is relatively uncommon but may result in significant morbidity and mortality. A major industrial use of EG is for synthesis of polyethylene terphthalate resins (PET) and fibers (polyester) in the bottling and clothing industries, while the major source of human toxicity (primarily from ingestion) is from additional uses of EG in household, automotive, and aircraft products including antifreeze, hydraulic brake fluids, and de-icing solutions. EG poisoning is characterized by a severe metabolic acidosis, cardiopulmonary complications, acute renal failure, and death. In 2007, the Association of Poison Control Centers reported 5731 EG exposures (84% unintentional) resulting in 2279 health facility treatments and 34 deaths (1). A medical examiner database that would better reflect the incidence of fatal EG poisoning is not readily available, but 5 years of regional data in a series of 4519 deaths investigated for drugs and chemicals by the Forensic Toxicology Laboratory at the Albany Medical Center revealed 12 fatalities for which EG was determined as the cause of death.

EG is metabolized (2) initially by alcohol dehydrogenase to glycoaldehyde with rapid subsequent conversion to glycolic acid (GA). GA is slowly converted to glyoxylic and then to oxalic acid, which precipitates as calcium oxalate in the kidney and in vascular endothelium of brain and other organs (3–10). Although EG toxicity is primarily the result of metabolic products, laboratory measurement of unmetabolized EG in blood remains the most frequently used method of confirming EG poisoning in both clinical and medical examiner practice. Many centers have reported EG measurement using direct (11–14) and derivatized (15–20) analysis by flame-ionization gas chromatography (GC–FID) as well as by rapid non-chromatographic techniques (21,22). Fewer methods for measurement of serum GA have been reported with testing confined to clinical application (6,23–27). For postmortem investigation, data is available on EG levels in fatal poisonings (28), but the concentration of GA in postmortem blood and its relationship to EG have not been reported. We have adapted and validated a GC–mass spectrometry (MS) method for use in postmortem blood investigation of EG poisoning. Application of the method in a series of EG fatalities is reported.

Experimental

Chemicals

EG, GA, diethylene glycol (DEG), propionic acid, 2,2-dimethylpropane (DMP), lactic acid, 1,3-propanediol (PD), (*S*)-(+)-1,2-propanediol, (*R*)-(+)-1,2-propanediol, (2*R*,3*R*)-(–)-2,3-butanediol, (2*S*,3*S*)-(+)-2,3-butanediol, and meso-2,3-butanediol were purchased from Sigma Aldrich (St. Louis, MO). Acetonitrile, ethyl acetate, diethylene glycol, 1,2-propanediol

(propylene glycol), ethyleneglycol monomethyl ether, ethyleneglycol monoethyl ether, and ethyleneglycol monobutyl ether were obtained from Fisher Scientific (Fairlawn, NJ). *N*,*N*-Dimethylformamide (DMF) was purchased from Burdick and Jackson (Muskegon, MI), and *N*-methyl-*N*-*t*-butyl dimethylsilyl trifluoroacetamide (MTBSTFA) was from Regis (Morton Grove, IL). Deionized water was produced by an ultrafiltration (AquaPure, Schenectady, NY) and reverse osmosis (Alpha Water Systems, Montague, NJ) treatment system.

Postmortem Study

Postmortem studies were conducted to determine the blood concentrations of EG and GA in 12 fatalities where EG poisoning was identified by the forensic pathologist as the cause of death. As a control group, EG and GA analysis was also performed with postmortem blood from 20 autopsy cases where EG exposure was not suspected and where a non-EG cause of death was determined by the examining pathologist. Under an Albany Medical College approved Institutional Review Board protocol, postmortem blood measurements of EG and GA by GC–MS were performed, and renal histology slides that were available from 10 of the 12 EG poisoning cases were re-examined and graded by polarized light microscopy. Birefringent crystal content was semiquantified as zero (none), 1+ (scattered), 2+ (moderate), or 3+ (large number).

Analytical

EG and GA were identified and quantified in blood by ion trap GC–MS analysis in scanning mode using a previously reported procedure (26) with a modified sample preparation and ion trap GC–MS analysis. Aliquots (50 µL) of centrifuged postmortem blood, along with matrix-matched calibrators and controls, were diluted with 50 µL of water and sonicated for 10 min in a Branson ultrasonic bath (Shelton, CT) prior to the addition of 150 µL of acetonitrile containing PD (500 mg/L) as internal standard. After centrifugation, 100 µL of supernatant was added to 500 µL of a water scavenger solution (DMP/DMF, 80:20, v/v) in a fume hood, then vortex mixed, and centrifuged. The supernatant was dried at 80°C, volume reduced to approximately 100 µL, and derivatized at room temperature by addition of 100 µL of MTBSTFA. Following derivatization, samples were diluted with 1 mL of ethyl acetate and injected (1 µL) onto the head of an analytical column in splitless mode. Chromatographic separation was performed with a Varian 3900 GC (Varian Chromatography Systems, Walnut Creek, CA) using a 5% diphenyl/95% dimethyl polysiloxane capillary column (Rxi-5ms, 15 m × 0.25-mm i.d., 0.25-µm film thickness, Restek, Bellefonte, PA) with a helium gas flow rate of 1 mL/min. A column temperature program with an initial 1-min hold (80°C) followed by temperature ramps of 80–125°C (25°C/min), 125–160°C (5°C/min), and 160–220°C (25°C/min) was employed. An injection temperature of 250°C and an ion trap temperature of 200°C were used. The GC was interfaced to a Saturn 2100T ion trap MS (Varian) operated in the electron impact mode of ionization with a mass spectral scan range of *m/z* 130–295. Identification of EG and GA by chromatographic and library match criteria with authenticated reference standards was performed by mass spectral analysis in scan mode. Quantification was performed using selective ions for EG (*m/z* 147, 233) and GA (*m/z* 147, 247) along with PD (*m/z* 247) as internal standard. Transition pathways and proposed molecular structures for the observed MS spectral ions of the EG and GA derivatives were evaluated by GC–MS–MS analysis. Following ionization, EG fragment ions (*m/z* 275, 233, 159, 147) and GA fragment ions (*m/z* 247, 163, 147) were individually isolated in a series of mass spectral experiments and further fragmented by resonant collision induced dissociation by application of an excitation amplitude of 0.6 volts followed by a mass ion scan for identification of primary transition ions. Workstation software version 5.52 (Varian) was used for instrument data management.

Quantification of EG was also performed by a wide-bore capillary GC–FID method described by Edinboro et al. (14), using direct injection of an ultra-filtered sample of centrifuged postmortem blood as adapted from the procedure of Cummings and Jatlow (12). One milliliter of blood, with the addition of 100 µL of 500 mg/L internal standard (DEG) was applied to Amicon Ultra-4 10000MWCO filter reservoir (Millipore, Billerica, MA) and centrifuged (3000 rpm) for 10 min, resulting in approximately 400–750 µL of ultrafiltrate. A 1-µL sample of the ultrafiltrate was injected onto the head of a fused silica wide-bore capillary column (Nukol, 30 m, 0.53-i.d., 0.50-µm film, Supelco, Bellefonte, PA) using an Auto System GC–FID (PerkinElmer, Norwalk, CT) and helium carrier gas (10 psi flow). A column temperature program with a temperature ramp of 110–180°C (8°C/min) was employed. The method demonstrated linear over an EG concentration range of 50–2000 mg/L. Interrun precision was evaluated at EG concentrations of 250 mg/L [standard deviation (sd) 13.5; percent coefficient variation (%CV) 5.44] and 750 mg/L (sd 39.2; % cv 5.25).

Results and Discussion

Ion trap GC–MS method validation

Total ion chromatograms for EG, GA, and internal standard derivatives are shown in Figure 1 along with inserts of MS spectra for the derivatized analytes and internal standard, as well as for the derivative of endogenous lactic acid. Scanning mode MS analysis allowed identification of the analytes based upon chromatographic and mass spectral matching criteria using experimentally determined library spectra for di-tBDMTMS derivatives of authenticated EG and GA reference material. The total ion chromatogram shows close elution of endogenous lactic acid following the internal standard (PD), but selective use of the *m/z* 247 as the quantification ion for PD eliminates potential interference from lactic acid. As further molecular confirmation of fragmentation ions resulting from MS analysis of the di-tBDMTMS derivatized analytes, Figure 2 and 3 show the transition pathway analysis by ion trap

GC–MS–MS along with proposed structures for EG and PG ions, respectively. Structurally different ion fragments of EG (e.g., *m/z* 233, 159) and GA (e.g., *m/z* 247, 163) are evident in this analysis and the specific ion transitions for EG (*m/z* 233 > 147; *m/z* 159 > 147) and GA (*m/z* 247 > 147; *m/z* 163 > 133) are identified for potential application in the development of further confirmatory testing by tandem MS analysis.

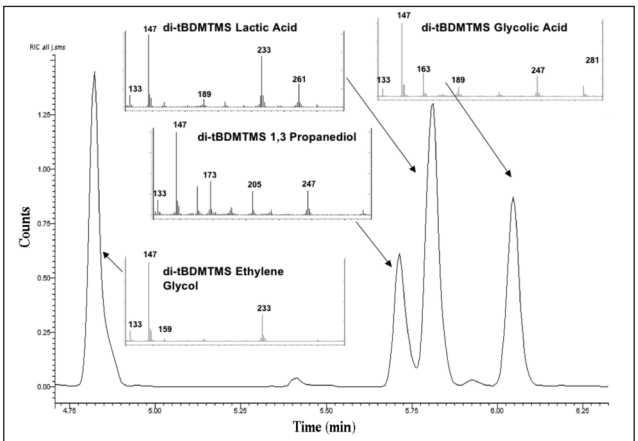

Figure 1. Total ion chromatogram of postmortem blood containing EG and GA. Inserts show mass spectra for the derivatized analytes, internal standard, and endogenous lactic acid.

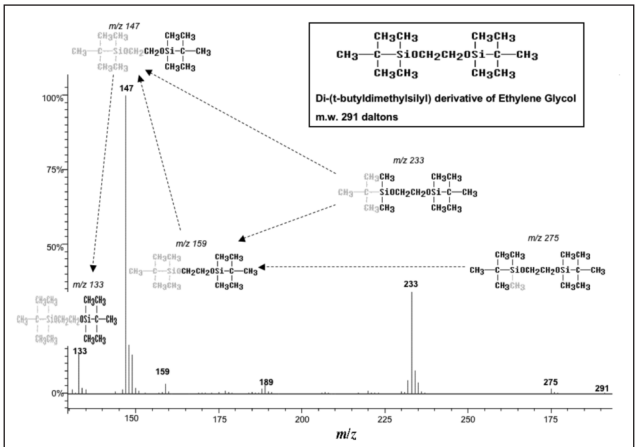

Figure 2. Transition pathway analysis by ion trap GC–MS–MS along with proposed ion fragment structures for EG derivatives. (Lost portion from each fragment denoted in gray.)

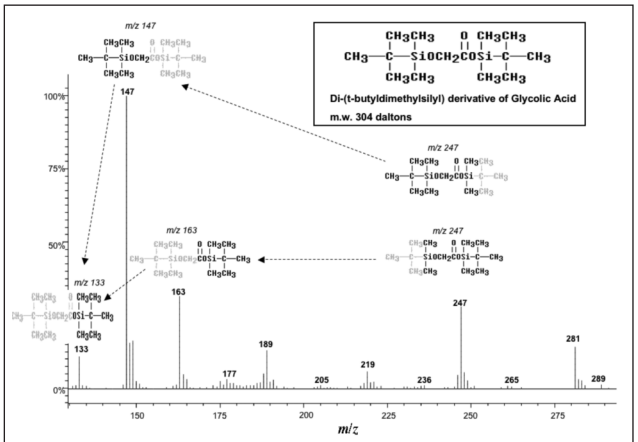

Figure 3. Transition pathway analysis by ion trap GC–MS–MS along with proposed ion fragment structures for GA derivatives. (Lost portion from each fragment denoted in gray.)

A minor modification of sample preparation involving initial sample dilution with sonication was employed in order to achieve linearity and recovery of GA quantification by ion trap GC–MS analysis. Figure 4 displays calibration data for a typical EG and GA analysis and shows linearity over the concentration range of 50–2000 mg/L. Validation studies also showed that additional sample dilution using a 25-µL aliquot of postmortem blood diluted with 75 µL of water prior to sonication extended the upper limit of quantitation to 4000 mg/L. A lower limit of detection of 25 mg/L was experimentally determined for both EG and GA, and each analytical run was challenged with quality control material at the detection limit concentration.

Recovery and precision were determined with control pool analysis performed in nine analytical runs. Recovery of EG and GA in postmortem blood is shown in Figure 5 with accuracy ranging from 96 to 101% and 92 to 108% for EG and GA, respectively. Interrun precision is profiled in Figure 6 with %CV ranges of 2.4–10.3 and 5.5–10.4 for EG and GA, respectively. Correlation of EG concentration by ion trap GC–MS with results of a GC–FID method is displayed in Figure 7 and demonstrates close agreement of the new method with a GC–FID method that has been used in our laboratory for many years.

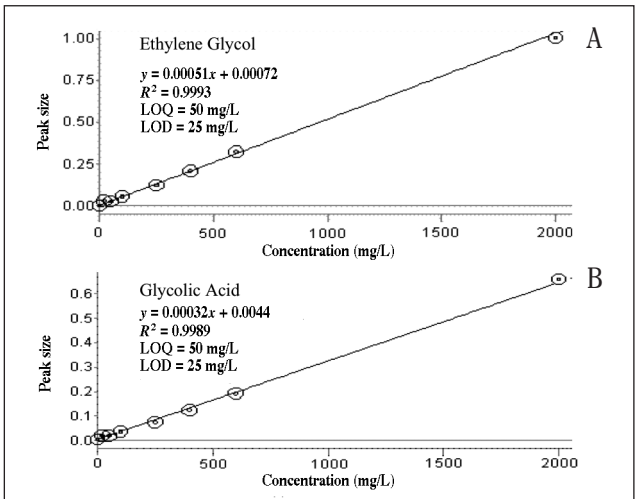

Figure 4. Calibration curves for EG (A) and GA (B) analysis with regression analysis results and limits of quantitation and detection.

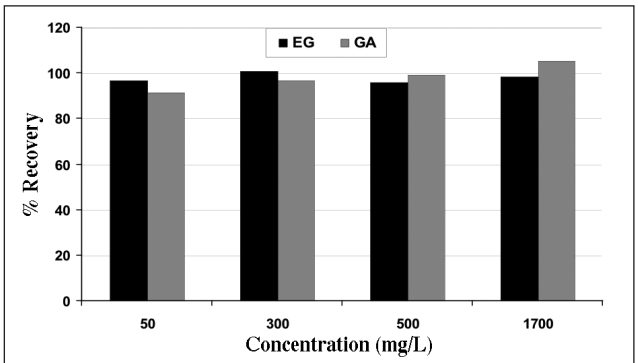

Figure 5. Recovery of EG and GA in postmortem blood.

Analysis of negative postmortem blood supplemented with 2500 mg/L of propionic acid, ethyleneglycol monobutyl ether, lactic acid, ethyleneglycol monomethyl ether, propylene glycol, ethyleneglycol monoethyl ether, diethylene glycol, formic acid, meso-2,3-butanediol, (*S*)-(+)-1,2-propanediol, (2*R*,3*R*)-(–)-2,3 butanediol, (*R*)-(+)-1,2-propanediol, and (2*S*,3*S*)-(+)-2,3 butanediol showed no interference by these agents in the measurement of EG and GA.

Postmortem case studies

Analysis of postmortem blood obtained at autopsy from 20 fatalities with no evidence of EG exposure and where causes of death other than ethylene glycol poisoning were determined demonstrated EG and GA concentration below the limit of detection of 25 mg/L. Figure 8 shows the results of EG and GA analysis in 12 fatal EG poisonings. EG concentration ranged widely from near the lower limit of quantitation to a highest reported value of 7790 mg/L with an average EG concentration of 1830 mg/L. GA levels ranged more narrowly from 810 to 1770 mg/L with a mean concentration of 1360 mg/L. Although similar data on co-measured EG and GA in postmortem cases are not available, our finding did compare well with studies of severely EG poisoned patients admitted for treatment in hospital facilities. In a fomepizole treatment trial, initial serum concentration of EG in 10 patients with EG poisoning averaged 1100 mg/L with a range of 50–3860 mg/L (8). Consistent with our findings in fatalities, the serum GA concentration in this clinical trial averaged 1290 mg/L with a range of 760–1800 mg/L. In another study of patient cohorts that either died or experienced acute renal failure from EG poisoning, the initial admission EG (80–810 mg/L) and GA (760–2890 mg/L) again showed the wide ranging EG levels with all GA levels exceeding 700 mg/L (29).

Discordant EG and GA concentration was evident in two of the decedents tested. The EG concentration was near the lower limit of quantitation (58 and 88 mg/L), and the corresponding GA concentrations of 1070 and 910 mg/L were highly elevated. Regression analysis of EG and GA levels for all cases showed lack of correlation with an R^2 of only 0.148. Discordantly low EG concentration has also been reported in clinical cases where concomitant acid base status was also measured along with EG and GA. In the fomepizole trial, a patient with severe metabolic acidosis evident by an initial blood pH of 7.13 and serum bicarbonate of 5.5 mmol/L had initial EG and GA determinations of 50 mg/L and 1480 mg/L, respectively (8). Our regression analysis performed with EG and GA data from this trial also demonstrated a lack of correlation (R^2 = 0.067). In another clinical report focusing on hemodialysis treatment for EG-intoxicated patients (26), EG and GA levels of 60 and 1100 mg/L, respectively, were reported in a patient in a state of severe metabolic acidosis (pH 6.89, bicarbonate 2 mmol/L, anion gap 29 mmol/L). Discordantly low or undetectable levels of EG are attributed to the rapid metabolism of EG coupled with the relatively long half-life and slow elimination rate of GA (8,9,24). Also reported in clinical studies, but not identified in our postmortem study, were individuals with elevated serum

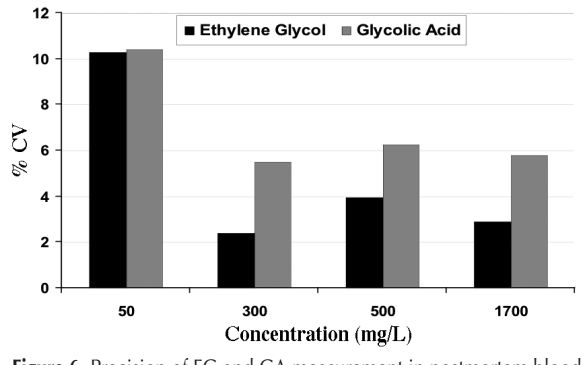

Figure 6. Precision of EG and GA measurement in postmortem blood.

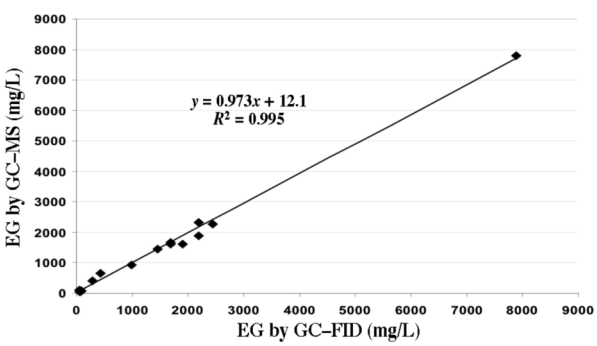

Figure 7. Comparison of EG concentration in postmortem blood between GC–MS and GC–FID methods of analysis.

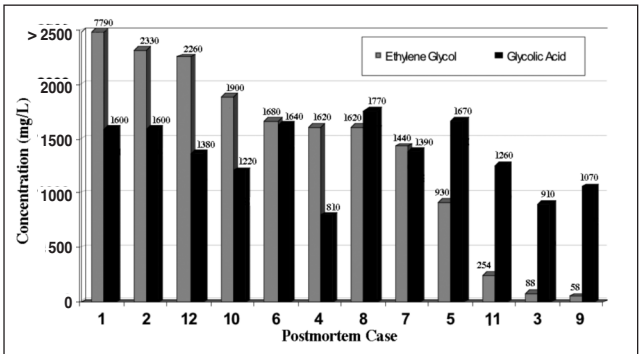

Figure 8. Concentration of EG and GA in postmortem blood from 12 cases of fatal EG poisoning. Cases sorted in order of decreasing EG concentration.

Table I. Detection and Grading of Birefringent Crystals in Renal Histology Sections From 12 Fatal Poisoning with EG

Case	Birefringent Crystal Grade
1	2+
2	1+
3	3+
4	2+
5	3+
6	2+
7	3+
8	3+
10	3+
12	2+

EG levels as high as 7100 mg/L but with GA levels below the detection limit (29). These patients who all survived experienced only limited signs of CNS toxicity without acidosis or acute renal failure, thus explaining the lack of this finding in a postmortem study. The postmortem and clinical studies, therefore, indicate that measurement of EG alone may not provide a sensitive index for detection or determination of severity of EG poisoning.

As the degree of metabolic acidosis, and not the level of EG, correlate with severity of outcome in EG-poisoned individuals (30), the level of GA in postmortem blood may provide an index of the degree of metabolic acidosis at the time of death. It is known that early inhibition of EG metabolism prevents metabolic acidosis and organ damage, and clinical studies have shown that GA concentration reflects the metabolic status and severity of intoxication (6,8–10,26,28,31–33). In a clinical study of 19 patients who were treated for the ingestion of EG, no renal dysfunction was found in the 10 patients with serum GA levels less than 760 mg/L (32). In a later clinical study with 39 patients, hemodialysis treatment was not recommended when the serum GA concentration was less than 630 mg/L, and further metabolism of EG was therapeutically inhibited (29). A direct relationship between increasing GA concentration and decreasing blood pH has been reported in both of the latter studies. The regressed relationship of pH and GA as reported by Porter and coworkers (29) and the GA levels measured in the postmortem cases in our study would indicate a severe acidosis in all 12 decedents at the time of death.

Unlike the clinical setting where direct acid base measurements are an index for continuing suspicion of EG poisoning when EG and GA measurement are not available, renal histology provides an anatomical index of suspicion in postmortem investigation. In the examination of kidney tissue from 10 decedents where tissue sections were available, all 10 cases revealed birefringent oxylate crystals with a grading of 1+ to 3+ (Table I). The finding is consistent with prior reports (34–38) and indicate sufficient post ingestion time for metabolism to oxalate and deposition in renal tissue prior to death. Although the presence of crystals was found in all cases, the semi-quantification of crystal content by grading did not correlate with the degree of elevation of either EG or GA concentration.

Conclusions

Analysis of postmortem blood using a validated ion trap GC–MS method for determination of EG and GA shows undetectable EG and GA levels in decedents without evidence of EG poisoning. In blood obtained at autopsy from 12 deaths caused by EG poisoning, EG (58–7790 mg/L) and GA (810–1770 mg/L) levels were determined with discordantly low EG concentration in two cases. The GA level in postmortem blood was consistent with clinical levels of serum GA in patients who experienced severe metabolic academia with outcomes including acute renal failure and death. Measurement of GA is recommended in the postmortem investigation of EG poisoning as EG determination alone may not provide a sensitive index for detection or determination of severity of EG poisoning. Birefringent oxylate crystals in kidney sections provided a consistent histological clue for EG poisoning in unsuspected cases.

References

1. A.C. Bronstein, D.A. Spyker, L.R. Cantilena, Jr., J.L. Green, B.H. Rumack, and S.E. Heard. 2007 annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 25th Annual Report. *Clin. Toxicol*. **46:** 927–1057 (2008).
2. G. Cruzan, R.A. Corley, G.C. Hard, J.J. Mertens, K.E. McMartin, W.M. Snelling, R. Gingell, and J.A. Deyo. Subclinical toxicity of ethylene glycol in Wistar and F344 rats related to metabolism and clearance of metabolites. *Toxicol. Sci*. **81:** 502–511 (2004).
3. K. Froberg, R.P. Dorion, and K.E. McMartin. The role of calcium oxalate crystal deposition in cerebral vessels during ethylene glycol poisoning. *Clin. Toxicol*. **44:** 315–318 (2006).
4. C. Guo, T.A. Cenac, Y. Li, and K.E. McMartin. Calcium oxalate, and not other metabolites, is responsible for the renal toxicity of ethylene glycol. *Toxicol. Lett*. **173:** 8–16 (2007).
5. D. Jacobsen and K.E. McMartin. Methanol and ethylene glycol poisoning. Mechanism of toxicity, clinical course, diagnosis and treatment. *Med. Toxicol*. **1:** 309–334 (1986).
6. P.A. Gabow, K. Clay, J.B. Sullivan, and R. Lepoff. Organic acids in ethylene glycol intoxication. *Ann. Int. Med*. **105:** 16–20 (1986).
7. D. Jacobsen, S. Øvrebø, J. Østborg, and O.M. Sejersted. Glycolate causes the acidosis in ethylene glycol poisoning and is effectively removed by hemodialysis. *Acta Med. Scand*. **216:** 409–416 (1984).
8. C.L. Moreau, W. Kerns, C.A. Tomaszewski, K.E. McMartin, S.R. Rose, M.D. Ford, and J. Brent. Glycolate kinetics and hemodialysis clearance in ethylene glycol poisoning. *J. Toxicol. Clin. Toxicol*. **36:** 659–666 (1998).
9. T.P. Hewlett, K.E. McMartin, A.J. Auro, and F.A. Ragan, Jr. Ethylene glycol poisoning, the value of glycolic acid determinations for diagnosis and treatment. *J. Toxicol. Clin. Toxicol*. **24:** 389–402 (1986).
10. B. Hylander and C.M. Kjellstrand. Prognostic factors and treatment of severe ethylene glycol intoxication. *Intensive Care Med*. **22:** 546–552 (1996).
11. R.O. Bost and I. Sunshine. Ethylene glycol analysis by gas chromatography. *J. Anal. Toxicol*. **4:** 102–103 (1980).
12. K.C. Cummings and P.I. Jatlow. Sample preparation by ultrafiltration for direct gas chromatographic analysis of ethylene glycol in plasma. *J. Anal. Toxicol*. **6:** 324–326 (1982).
13. J.A. Jonsson, A. Eklund, and L. Molin. Determination of ethylene glycol in postmortem blood by capillary gas chromatography. *J. Anal. Toxicol*. **13:** 25–26 (1989).
14. L.E. Edinboro, C.R. Nanco, D.M. Soghioan, and A. Poklis. Determination of ethlene glycol in serum utilizing direct injection on a wide-bore capillary column. *Ther. Drug Monit*. **15:** 220–223 (1993).
15. D.W. Robinson and D.S. Reive. A gas chromatographic procedure for quantitation of ethylene glycol in postmortem blood. *J. Anal. Toxicol*. **5:** 69–72 (1981).
16. H.H. McCurdy and E.T. Solomons. An improved procedure for determination of ethylene glycol in blood. *J. Anal. Toxicol*. **6:** 253–254 (1982).
17. W.H. Porter and A. Auansakul. Gas-chromatographic determination of ethylene glycol in serum. *Clin. Chem*. **28:** 75–78 (1982).
18. M. Balikova and J. Kohlicek. Rapid determination of ethylene glycol at toxic levels in serum and urine. *J. Chromatogr*. **434:** 469–474 (1988).
19. W.H. Porter, M.C. Jarrells, and D.H. Sun. Improved specificity for ethylene glycol determination as the phenylboronate by capillary

column gas chromatography. *Clin. Chem*. **40:** 850–851 (1994).
20. Y.M. Pan, G.N. Gill, C.S. Tilson, W.H. Wall, and H.H. McCurdy. Improved procedure for the analysis of gamma-hydroxybutyrate and ethylene glycol in whole blood. *J. Anal. Toxicol*. **25:** 328–332 (2001).
21. P. Hansson and P. Masson. Simple enzymatic screening assay for ethylene glycol (ethane-1,2-diol) in serum. *Clin. Chim. Acta* **182:** 95–101 (1989).
22. J.M. Meola, T.G. Rosano, and T.A. Swift. Fluorometry of ethylene glycol in serum. *Clin. Chem*. **26:** 1709 (1980).
23. T.P. Hewlett, A.C. Ray, and J.C. Reagor. Diagnosis of ethylene glycol (antifreeze intoxication in dogs by determination of glycolic acid in serum and urine with high performance liquid chromatography and gas-chromatography–mass spectrometry. *J. Assoc. Anal. Chem*. **66:** 275–283 (1983).
24. A.D. Fraser and W. MacNeil. Colorimetric and gas chromatographic procedures for glycolic acid in serum: the major toxic metabolite of ethylene glycol. *J. Toxicol. Clin. Toxicol*. **31:** 397–405 (1993).
25. H.H. Yao and W.H. Porter. Simultaneous determination of ethylene glycol and its major toxic metabolite, glycolic acid, in serum by gas chromatography. *Clin. Chem*. **42:** 292–297 (1996).
26. W.H. Porter, P.W. Rutter, and H.H. Yao. Simultaneous determination of ethylene glycol and glycolic acid in serum by gas chromatography–mass spectrometry. *J. Anal. Toxicol*. **23:** 591–597 (1999).
27. M. Lovrić, P. Granić, M. Ćubrilo-Turek, Z. Lalić, and J. Sertić. Ethylene glycol poisoning. *Forensic Sci. Int.* **170:** 213–215 (2007).
28. R.C. Baselt. Ethylene glycol. In *Disposition of Toxic Drugs and Chemicals in Man*, 8th ed. Biomedical Publications, Foster City, CA, 2008, pp 578–582.
29. W.H. Porter, P.W. Rutter, B.A. Bush, A.A. Pappas, and J.E. Dunnington. Ethylene glycol toxicity: the role of serum glycolic acid in hemodialysis. *Clin. Toxicol*. **39:** 607–615 (2001).
30. C. Karlson-Stiber and H. Persson. Ethylene glycol poisoning: experiences from epidemic in Sweden. *J. Toxicol. Clin. Toxicol*. **30:** 565–574 (1992).
31. K.C. Clay and R.C. Murphy. On the metabolic acidosis of ethylene glycol intoxication. *Toxicol. Appl. Pharmacol*. **39:** 39–49 (1977).
32. J. Brent, K. McMartin, S. Phillips, K.K. Burkhart, J.W. Donovan, M. Wells, and K. Kulig. Fomepizole for the treatment of ethylene glycol poisoning. *N. Engl. J. Med*. **340(11):** 832–838 (1999).
33. A.D. Fraser. Clinical toxicologic implications of ethylene glycol and glycolic acid poisoning. *Ther. Drug Monit*. **24:** 232–238 (2002).
34. S. Takahashi, J. Kanetake, Y. Kanawaku, and M. Funayama. Brain death with calcium oxalate deposition in the kidney: clue to the diagnosis of ethylene glycol poisoning. *Legal Med*. **10:** 43–45 (2008).
35. C. Pomara, C. Fiore, S. D'Errico, I. Riezzo, and V. Fineschi. Calcium oxalate crystals in acute ethylene glycol poisoning: a confocal laser scanning microscope study in a fatal case. *Clin. Toxicol.* **46:** 322–324 (2008).
36. E.J. Armstrong, D.A. Engelhart, A.J. Jenkins, and E.K. Balraj. Homocidal ethylene glycol intoxication: a report of a case. *Am. J. Forensic Med. Pathol*. **27:** 151–155 (2006).
37. P.M. Leth and M. Gregersen. Ethylene glycol poisoning. *Forensic Sci. Int*. **155:** 179–184 (2005).
38. S. Siew. Investigation of crystallosis of the kidney by means of polarization, scanning, transmission and high-voltage electron microscopy. *Isr. J. Med. Sci*. **15:** 698–710 (1979).