Identification and Quantification of Diethylene Glycol in Pharmaceuticals Implicated in Poisoning Epidemics: An Historical Laboratory Perspective

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Abstract

Over the last several decades, mass poisonings of diethylene glycol (DEG), usually ingested as an unintended component of pharmaceutical preparations, have occurred. In order to promptly halt the rise in deaths due to ingestion of these pharmaceuticals, laboratory analysis has often been employed to identify and quantify the etiologic agent after the medications have been tentatively implicated. Over the past 15 years, the Centers for Disease Control and Prevention has been involved in identifying DEG in implicated pharmaceutical products during three poisoning epidemics that occurred in Nigeria (1990), Haiti (1995), and, most recently, in Panama (2006). In each case, the timeliness of the identification was paramount in reducing the mortality involved in these mass poisonings. Using state-of-the-art analytical technology, we were able to provide initial identification of DEG within 24 h of receipt of samples for each epidemic, allowing a timely public health response. However, over the past 15 years, the analytical instrumentation available and the laboratory responses undertaken have changed. In addition, the type of information and the degree of confirmation of results requested during each epidemic varied based upon the number of individuals involved and the political tenor involved with the outbreak. We describe our historical approach to identifying and quantifying DEG during each of these outbreaks. Furthermore, the reoccurrence of outbreaks has prompted us to establish standard technology to use in potential future outbreaks to allow an even more timely response. This methodology includes the development of biomarkers of DEG exposure, which would be extremely useful in instances where pharmaceuticals are not clearly implicated.

Introduction

Over the course of the last century, many episodes of mass poisoning from ingestion of diethylene glycol (DEG; 2,2'-oxybisethanol; CAS# 111-46-6) have occurred (1). DEG is a four-carbon dimer of ethylene glycol, a solvent used in antifreeze. Although the acute toxicity of ethylene glycol was known before the 1930s, the toxicity of DEG had not been documented prior to the first instance of mass poisoning (2). The first incident of mass poisoning by DEG, known as the Massengill incident, uncovered similar symptoms of toxicity between ethylene glycol and DEG (1,2). The Massengill incident occurred in 1937 when a sulfanilamide drug was prepared with DEG. After consumption of this drug, 105 adults and children died. This prompted the passage of the Food, Drug and Cosmetic Act of 1938, which was to oversee the safety of products entering the U.S. market (2). After this initial incident where DEG was intentionally used as the diluent in a pharmaceutical product, several other instances have occurred, primarily in developing countries, where DEG was mistakenly used as a diluent instead of more common diluents such as propylene glycol and glycerin (1,3,12). These occurrences are outlined in Table I.

Over the past 15 years, the Centers for Disease Control and Prevention (CDC) has been involved in identifying DEG in implicated pharmaceutical products during the poisoning epidemics that occurred in Nigeria (1990) (11), Haiti (1995) (7), and, most recently, in Panama (2006). In each case, the timeliness of the identification was paramount in reducing the mortality involved in these mass poisonings. Using state-of-the-art analytical technology, we were able to provide initial identification of DEG within 24 h of receipt of samples for each epidemic, allowing a timely public health response. However, over the past 15 years, the analytical instrumentation available and the laboratory responses undertaken have changed. In addition, the type of information and the degree of confirmation of results requested during each epidemic varied based upon the number of individuals involved and the political tenor involved with the outbreak. We describe our historical approach to identifying and quantifying DEG during each of these outbreaks.
Nigeria Incident

Nigeria is located in West Africa and the most populous country in Africa with a total census of over 130 million residents. The residents of Nigeria were in an environment of volatility and strife through 1999 while being ruled by a series of military dictators, and despite the restoration of democratic rule, the economy has continued to suffer. Health, health care and general living conditions in Nigeria are poor. The life expectancy is 47 years, and just over half the population has access to potable water and appropriate sanitation. Infant mortality is about 10% of live births, and death clusters in children are only noticed if they are large.

During the summer months of 1990, approximately 47 children were admitted to the Jos University Teaching Hospital with symptoms including anuria, fever, and vomiting (11). All children had fever prior to developing acute renal failure. Attending physicians quickly identified a paracetamol syrup that was in common with the majority of the patients.

Experimental

Five samples of paracetamol syrup collected from the patients' families and one control sample were shipped to CDC for identification of the etiologic agent. Approximately 2 mL of each sample was obtained, and each sample was shipped in polypropylene vials rather than in their original containers. At

![Figure 1](image)

Figure 1. Accurate mass measurement of paracetamol using fast atom bombardment-mass spectrometry in the Nigeria incident. The sodium adduct of an implicated syrup and DEG standard are shown in A and C, respectively (accurate mass = m/z 129.0528). The protonated DEG in an implicated syrup and DEG standard are shown in B and D, respectively (accurate mass = 107.0708).
this time, current technologies such as mass spectrometers (MS) interfaced with liquid chromatographs (LC) were not available for use in identification and quantification. Using a VG70-4SE double-focusing tandem magnetic sector MS (Micromass, Manchester, England), the samples were dissolved in a "magic bullet" matrix (dithiothreitol/dithioerythritol) and analyzed by accurate mass fast atom bombardment (FAB)-MS. The ions were referenced to the exact masses of the matrix ions. No further confirmation or any quantification was performed.

Results and discussion

DEG was detected in all of the implicated samples but not in the control sample. The accurate mass measurements were referenced against known matrix ions instead of the usual polyethylene glycols (PEGs) because DEG would be expected to be present in small amounts in PEGs. Deviations in the accurate mass measurements from the calculated mass of both the molecular ion and the sodium adduct of DEG in case-associated samples ranged from 5.2 to 7 ppm (Figure 1). Accurate mass measurements within 10 ppm of the calculated mass are considered confirmatory. DEG standards produced similar results although their deviations from the accurate mass were somewhat smaller (1.2 to 4 ppm).

Although no raw materials were tested or quantified, the presence of DEG in the pharmaceutical syrups was assumed to be an unintentional substitution of a portion of the propylene glycol diluent with DEG. This incident served as an impetus for the Nigerian government to develop standard guidelines for quality control of pharmaceutical products.

Haiti Incident

Haiti is a poor country, the poorest in the Western Hemisphere, and approximately 85% of the nearly 7 million residents there live in abject poverty. Given this backdrop, and the frequency of illness and death in children, clusters of disease may not be recognized unless they are large or unusual. The first two children with acute renal failure were admitted to the University Hospital in Port-au-Prince in November of 1995. Later, more patients would be admitted with the same syndrome. All of these children were less than 18 years of age, nearly all had a history of fever, and, most notably, the case fatality rate approached 100%. By June 1996, the number of cases and suspected cases approached 50 children and CDC was invited to conduct an epidemiologic investigation (7).

Early in the investigation, epidemiologists discovered that most patients had consumed one of two acetaminophen preparations, Afebril or Valodon, which were both manufactured by the same pharmaceutical company (7). Inspection of the pharmaceutical company uncovered poor quality control procedures and an unclean production facility. Politics were a driving factor in the extent of this investigation because of the strong relationship between the pharmaceutical manufacturer and the Haitian government.

Experimental

Initial identification. On June 21, 1996, two samples of Afebril and Valodon collected from patient families were sent to CDC for analysis. Our laboratory was able to quickly identify the etiologic agent in the medications as DEG. For initial identification, we diluted the syrups 1:50 with methanol. The dilute samples were analyzed by flow injection-positive electrospray ionization (FIA-ESI)-MS using a VG70-4SE double-focusing tandem magnetic sector MS (Micromass). The solvent system consisted of 0.1% aqueous acetic acid/methanol (1:1) at a flow rate of 0.5 mL/min delivered by an HP 1090L high-performance liquid chromatograph (HPLC, Hewlett-Packard, Santa Clara, CA). Using the same instrument, the samples were dissolved in a "magic bullet" matrix (dithiothreitol/dithioerythritol) and analyzed by accurate mass FAB-MS. The ions were referenced to the exact masses of the matrix ions.

The same samples were also analyzed by FIA-MS with positive atmospheric pressure electrospray ionization (+ESI) using a TSQ 7000 triple-quadrupole MS (Finnigan MAT, San Jose, CA). The solvent system consisted of 0.1% ammonium acetate buffer/methanol (1:1) at a flow rate of 100 l/min delivered by an HP 1090L HPLC (Hewlett-Packard). The MS was operated in the full scan mode, and the electrospray voltage was operated at 5 kV. The capillary temperature was set at 200°C, and nitrogen was used as a sheath gas at 40 psi. An MS–MS confirmation was performed using the same instrument and conditions with argon (2 mT) as the collision gas and a collision offset of –28 V. To verify that any detectable DEG was not a degradation product of higher molecular weight PEGs, parent ion scans were also performed.

Quantitative analysis. After positively confirming DEG contamination of the syrups, epidemiologists instigated the appropriate interventions that included the passive and active recall of the implicated medications, which resulted in a dramatic drop in the epidemiologic curve. As the investigation ensued, shipments containing 2 to 20 medications were obtained for the next 3 weeks. Most of the samples had been retrieved from the victims' families and were in their original bottles; however, the bottles had been previously opened. The volumes of the medications in the bottles ranged from several microliters to about 200 mL.

More specific information regarding the implicated medications was requested by both the pharmaceutical manufacturer and the Haitian Minister of Health. Identification of the specific production lots that were contaminated and the quantification of the percentage of DEG in the contaminated syrups were requested. In response, we rapidly developed a specific method for identifying and quantifying DEG in the syrups.

For a control sample, a pediatric acetaminophen preparation manufactured in the United States (Tempra2, Mead Johnson and Co., Evansville, IN) was used. Pure DEG (99+%) was obtained from Fluka (St. Louis, MO). Deuterated DEG (d10, 99.9% isotopic purity) was donated by Cambridge Isotope Laboratories (Andover, MA).

The samples were prepared by dissolving 100 µL unknown syrup, control syrup, or DEG standard and 10 µL deuterated DEG in 4 mL methanol. The samples were mixed and equilibrated for approximately 30 min to allow adequate time for the
deuterium atoms on the hydroxyl groups of the labeled DEG to exchange with protons in the matrix.

The samples were analyzed by isotope-dilution HPLC–MS–MS using a TSQ-7000 triple-quadrupole MS (ThermoFinnigan, San Jose, CA) interfaced to an HP1090L HPLC via an atmospheric pressure ESI source. A 5-μ Absorbosphere C18 pre-column (4.6-mm i.d., Alltech, Deerfield, IL) was inserted between the injector and MS to improve sample peak shape. The solvent system was 1:1 water/methanol with 0.1% ammonium acetate and was delivered at a flow of 200 μL/min. The electrospray voltage and capillary and vaporizer temperatures were operated at 5 kV, 200°C, and 400°C, respectively. The nitrogen sheath gas was at 40 psi. The MS was operated in the positive product ion mode with 2 mT argon collision gas and a collision offset of -28 V. The precursor ions were the ammoniated species of the monomers, dimers, and mixed dimers of the native and labeled DEG (Table II). The product ion transformations were induced by multiple low energy collisions with argon gas. The product ions monitored were the pseudo-molecular ions of DEG [(M+H)+] at m/z 107 and labeled DEG at m/z 115. A calibration plot was constructed by plotting the response factors from multiple analyses against the concentration (expressed as a percentage of the formulation, v/v) of five standards (the equivalent of 10, 25, 50, 75, and 100% diethylene glycol in the formulation as spiked into the control syrup). The response factor was calculated as the ratio of the area of DEG to DEG-d8. A linear regression analysis of the data produced an equation that was used to quantify the percentage of diethylene glycol in the acetaaminophen syrups.

The method limit of detection (LOD) was determined as 3s0, where s0 is the signal at zero concentration. The s0 was estimated as the y-intercept of a linear regression analysis of a plot of the absolute standard deviation versus the concentration.

The accuracy of the method was validated by performing spiked recovery analyses. DEG was spiked into control acetaaminophen syrup at three different concentrations. The syrups were diluted, equilibrated, and analyzed. A linear regression analysis of a plot of expected concentration versus calculated concentration was performed. A slope of 1 is indicative of 100% accuracy.

We used this method to identify and quantify DEG in over 200 pharmaceutical products that included both implicated and non-implicated medications that were retrieved from the victims’ families. Additionally, we analyzed raw materials, quality control samples, and other pharmaceutical preparations provided by the medications’ manufacturer.

## Results and discussion

Using FIA-ESI-MS, we were able to detect the sodium adduct of DEG at m/z 129. In addition, we observed the sodium adduct of the dimer at m/z 235 and the sodium adduct of acetaminophen at m/z 173. These ions, with the exception of the sodium adduct of acetaminophen, were consistent with an authentic standard of DEG. Similarly, all ions, with the exception of the sodium adduct of acetaminophen, were absent from our control acetaminophen preparation. Sodium adduct ions were not unexpected as the pharmaceutical products contained a high content of sodium.

To confirm the identity of the observed masses, MS–MS was performed on the samples and standards; however, the sodium adduct formation was shifted to an ammonium adduct by the use of an ammonium salt in the mobile phase buffer (Figure 2). The standard DEG characteristically fragmented to produce ions at m/z 107, 89, 63, and 45 (Figure 3A). These masses are consistent with the molecular ion, loss of OH, loss of -CH2CH2OH, and loss of -OCH2CH2OH, respectively. MS–MS performed on the case-associated samples showed the same

### Table II. Analytical Conditions for the High-Performance Liquid Chromatography–Tandem Mass Spectrometry Quantification of Diethylene Glycol in Two Mass Poisoning Outbreaks

<table>
<thead>
<tr>
<th>Incident</th>
<th>Technique</th>
<th>Column</th>
<th>Mobile Phase System</th>
<th>Ion Type</th>
<th>Precursor Ion (m/z)</th>
<th>Product Ion (m/z)</th>
<th>Collision Offset (V)</th>
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<tbody>
<tr>
<td>Haiti*</td>
<td>HPLC–+ESI–MS–MS</td>
<td>C18 precolumn (5 μ; 4.6-mm i.d.)</td>
<td>isocratic: 1:1</td>
<td>NH4 adduct 124</td>
<td>107</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>water/methanol</td>
<td>NH4 adduct 132</td>
<td>115</td>
<td></td>
<td>28</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>with 0.1% ammonium acetate</td>
<td>ISTD dimer 212</td>
<td>107</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>ISTD dimer 228</td>
<td>115</td>
<td></td>
<td>28</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mixed dimer 238</td>
<td>107, 115</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>Panama*</td>
<td>HPLC–+APCI–MS–MS</td>
<td>SeQuant ZIC-HILIC</td>
<td>isocratic: 84.5%</td>
<td>DEG 107</td>
<td>45</td>
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<td>-10</td>
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<td>column, particle size 5 μm, 150 x 2.1 mm</td>
<td>acetonitrile and ammonium acetate</td>
<td>DEG ISTD 115</td>
<td>97</td>
<td></td>
<td>-12</td>
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<td></td>
<td></td>
<td></td>
<td>15.5% with 5mM</td>
<td>NH4 adduct 124</td>
<td>45</td>
<td></td>
<td>-12</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>ammonium acetate</td>
<td>ISTD NH4 adduct 132</td>
<td>115</td>
<td></td>
<td>-10</td>
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<td>ISTD ACN adduct 156</td>
<td>115</td>
<td></td>
<td>-7</td>
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</table>

* Quantification was achieved using all m/z 107 as the native DEG and all m/z 115 as DEG-d8 because of the variability in generating each species; however, all species had to be present for positive identification.

1 Abbreviations: ACN, acetonitrile; +APCI, positive atmospheric pressure chemical ionization; +ESI, positive electrospray ionization; HPLC, high-performance liquid chromatography; i.d., internal diameter; ISTD, labeled diethylene glycol (d8); MS–MS, tandem mass spectrometry; V, voltage; and m/z, mass-to-charge ratio.

2 Dimers were not observed under these analytical conditions.
characteristic fragmentation (Figure 3B). Additionally, the control sample did not show this fragmentation.

To confirm the mass observed at \( m/z \) 124 was not a source fragmentation product of PEGs, a common component of pharmaceutical preparations, parent ion MS-MS was performed. The data indicated that the only parent ion of \( m/z \) 124 was itself, thus confirming that it did not result from PEGs.

The accurate mass measurements were referenced against known matrix ions instead of the usual PEGs because DEG would be expected to be present in minute amounts in PEGs. Deviations in the accurate mass measurements from the calculated mass of both the molecular ion and the sodium adduct of DEG in case-associated samples ranged from 5 to 9 ppm. As mentioned earlier, accurate mass measurements within 10 ppm of the calculated mass are considered confirmatory. DEG standards produced similar results.

Full scan HPLC-MS spectra of many of the case-associated samples and the DEG standard showed that many dimers and ammonium adducts of DEG were formed, but not consistently. To better facilitate the quantification of DEG, we monitored the product ion transformations for both DEG and DEG-d8 of the monomer adducts, dimers, and mixed dimer adduct (Table II, Figure 4).

The \( R^2 \) value of a linear regression analysis of the calibration plot was 0.93 with less than 1% error about the slope. Using the data on this plot, the method LOD was determined to be 0.7% DEG (v/v) and the average relative standard deviation of the method was 16%. The calculated relative recovery (accuracy) was 98%.

The percentages of DEG in various lots of acetaminophen preparations manufactured in Haiti are shown in Figure 5. "C" lots were acetaminophen syrups for small children and "A" lots were acetaminophen infant drops. The average DEG contamination in "C" and "A" lots was 16 and 5.8%, respectively. Later "C" lots did not contain detectable levels of DEG. Interestingly, bottles of the "C3" lot were stamped with two different dates. The earlier dated bottles contained an average 16% DEG, but the later dated lots did not contain detectable levels of DEG.
It appears as if the actual preparation lot had changed but the lot numbers were not changed on the bottles.

Other preparations from the manufacturer's lot were tested along with many of the raw materials. DEG was found in only one other type of pharmaceutical preparation, vitamin C drops, but at much lower levels (1.2%). Glycerin, which was used in the syrup preparation, was contaminated with 24% DEG.

At the perceived end of the epidemic, at least 88 children died, and 8 recovered after extensive dialysis treatment in the United States.

Panama Incident

The Republic of Panama is the southernmost country in Central America with the lowest population in Spanish-speaking Latin America. Panama’s economy is service-based, heavily weighted toward banking, commerce, and tourism, because of its key geographic location. Because of its good economic conditions, it is a bastion for immigration and has become a melting pot, including many Caribbean and Asian immigrants. In general, the standard of living of residents is good.

In October 2006, CDC epidemiologists were invited to Panama to investigate an unusual cluster of acute renal failure in primarily elderly patients (13). Initial observations suggested a link between the hypertension medication Lisinopril, which is an ACE inhibitor. Analyses by the U.S. Food and Drug Administration indicated that the Lisinopril was uncompromised. A follow up of this initial link found that many of the patients taking Lisinopril had been prescribed an elixir to treat a chronic cough, a typical side effect of ACE inhibitors (13).

Samples of these elixirs and biological samples (urine, whole blood, serum, and cerebrospinal fluid) were collected and sent via chartered jet to CDC for analysis. After routine analyses for a series of metals in blood (R. Jones, personal communication) and pesticides or their metabolites in urine (14,15) provided no insight into the outbreak, we focused on DEG as the most probable etiologic agent.

Experimental

Initial identification. On October 10, 2006, 13 liquid medicines in their original containers were received in our laboratory for analysis. Of these medications, five were labeled as antihistamine/expectorant, two were expectorants, three were antacids, and one was a vitamin preparation. The last sample was shipped in a Falcon tube, not in its original container, but was obtained from one of the patient’s family members.

For primary identification of DEG, all samples were diluted 1:10 with methanol/water (1:10) prior to testing. Sources for DEG and labeled DEG were the same as in the Haiti incident. A CVS Pharmacy brand Tussin DM cough formula was used as a blank control. After spiking, the Tussin DM cough syrup served as a positive control. Samples were analyzed using HPLC–positive atmospheric pressure chemical ionization (APCI)-MS–MS in the product ion mode with four precursor to product ion pairs monitored with precursor ions including the parent DEG, and the ammonium and acetonitrile adducts (Table II). The HPLC–MS–MS analysis was performed on a Surveyor LC (Thermo Electron) interfaced to a Quantum triple-quadrupole MS (Thermo Electron). After injection, the analytes were chromatographically separated using a SeQuant ZIC-HILIC column (150 x 2.1 mm, 5-μm particle size, The Nest Group, Southboro, MA). The column was held constant at 35°C. The mobile phase was isocratic at a flow rate of 200 μL/min. The mobile phase was pre-mixed and consisted of 84.5% acetonitrile and 15.5% water, which were both modified with ammonium acetate (5mM). DEG eluted from the column at 2.59 min. The MS was operated in the selected reaction monitoring (SRM) mode using +APCI. Nitrogen was used for sheath gas (50 psi) and auxiliary gas (5 psi), and argon was used for CID gas (1.0 mTorr). Other optimized parameter values are listed in Tables II and III.

Samples were also analyzed using three other confirmatory techniques: Fourier transform (FT)-MS to provide accurate

<table>
<thead>
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<th>Parameter</th>
<th>Value</th>
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<tr>
<td>Discharge current</td>
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<tr>
<td>Vaporizer tempera</td>
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</tr>
<tr>
<td>Sheath gas pressure</td>
<td>50 psi</td>
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<tr>
<td>Capillary tempera</td>
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<tr>
<td>Tube lens offset</td>
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<tr>
<td>Source CID</td>
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<tr>
<td>Quad MS–MS bias</td>
<td>-2.2 eV</td>
</tr>
<tr>
<td>APCI probe position</td>
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</tr>
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</table>

* Abbreviations: APCI, atmospheric pressure chemical ionization; CID, collision-induced dissociation; eV, electron volts; mA, milliamperes; MS–MS, tandem mass spectrometry; and Quad, quadrupole.
mass measurements and gas chromatography—time of flight (GC–TOF)-MS and two-dimensional GC (GC×GC)—TOF-MS, in which all ions present in the sample could be monitored concurrently. For the FT-MS analysis, samples were diluted 1:100 in a solution of 70:30 water/acetonitrile with 0.1% formic acid and 1mM ammonium acetate. One-hundred microliters of each sample was directly infused at 300 nL/min into a 7-T LTQ-FT MS (Thermo Electron) utilizing a standard nanoelectrospray ion source interface. The FT-MS was used for MS acquisition with a resolution of 100,000 (defined at full width, half height) from m/z 50 to 200. The linear ion trap was used for MS–MS analysis of the precursor ion of interest. Automatic gain control was used to accumulate ions for FT-MS analysis with a target value of 1,000,000 and a target value of 2000 for MS–MS analysis. A collision energy of 20% was used in the linear ion trap to fragment the precursor ion. All spectra were a sum of 25 scans.

All samples were diluted 1:100 with methanol for the TOF-MS analyses. For the GC×GC–TOF-MS analyses, the first dimension column was a DB-5HT (5 m, 0.25-mm i.d., 0.10-µm phase thickness, J&W Scientific, Folsom, CA), and the second dimension column was a HT-8 (5 m, 0.25-mm i.d., 0.10-µm phase thickness, SGE Analytical Services, Austin, TX). The GC oven was programmed from 100°C (held 1 min) to 300°C with a ramp rate of 10°C/min. The final temperature was held for 5 min. The secondary column was operated with a 20°C offset from the primary oven. Spectral data were obtained with a 35 Hz resolution from m/z 50 to 1000.

For the GC–TOF analyses, a CP-WAX column (10 m, 0.1-mm i.d., 0.2-µm phase thickness, Varian) was used for separation. The GC oven was programmed from 70°C (held 1 min) and then ramped at 30°C/min to 250°C. The final temperature was held for 10 min. Spectral data were obtained with a 20 Hz resolution from m/z 30 to 350.

The instrumental analysis was conducted using a Leco Pegasus 4D TOF-MS (Leco Corp., St. Joseph, MI). The filament bias for the TOF-MS was 70eV.

The spectra were processed with an automated data processing method to identify and de-convolute co-eluting peaks. Processed data were reviewed manually. Automatic library searches were included in the data processing method and a maximum of 100 peaks for GC×GC–TOF-MS and a maximum of 10 peaks for GC-TOF were used for library searching.

Quantification. All implicated samples and raw glycerin diluent obtained from the pharmaceutical manufacturer were quantified using HPLC–MS–MS as described in the initial identification above with a few minor changes.

An isotopically labeled internal standard (ISTD) of diethylene glycol (DEG) (diethyl-d10) was purchased from Cambridge Isotope Laboratories with isotopic purity of 98%. An ISTD solution was prepared by adding 200 µL of isotopically labeled DEG into a 5-mL volumetric flask and diluting with purified water up to 5 mL (making 40 µL/mL concentration solution). This step would allow equilibration of the deuterium atoms on the hydroxyl groups with the protons in the water. Twenty microliters of ISTD solution was added to each sample (1:100 diluted cough syrup resulting in 1 mL sample) sample to obtain an ISTD concentration of ISTD of 0.8 µL/mL in each sample.

All solvents used were analytical grade with purity greater than 98%. Water was purified with a Nanopure Infinity system (Barnstead International, Dubuque, IA). Ten sets of calibration spiking standards (5 sets high and 5 sets low) in purified water with analyte concentrations ranging from 0.064 to 1600 mg/L (0.02 to 50% DEG, v/v) were prepared using serial dilution. For quantification, the value calculated using the isotope dilution calibration plot was multiplied by a factor of 100 to account for the initial dilution step.

The samples were prepared by adding 200 µL of cough syrup (implicated syrup, raw glycerin product, or calibrant) into a vial and diluting with 19.8 mL of purified water (making 1:100 dilution). The samples were mixed for 30 s. A 1-µL aliquot of diluted sample was taken, and 20 µL of ISTD solution was added to each sample. All the samples were mixed again to equilibrate the ISTD with the sample, and 100 µL of each sample was transferred to HPLC vials for the analysis. One microliter of each sample was injected to the LC column to analyze the concentration of DEG in cough syrup.

Accuracy, precision, and LODs were calculated in a manner similar to the Haiti incident.

Results and discussion
Each sample that tested positive contained all precursor-product ion pairs and chromatographically eluted at the same time as authentic DEG. One sample which tested positive was confirmed using FT-high resolution MS with accurate mass. In this sample, both the full spectrum ions and products ions provided the same masses as authentic DEG. All medications found to contain DEG were red colored antihistamine/expectorants produced by the same manufacturer with the same lot number. All positive and negative findings were reconfirmed by repeat analysis.

For FT-MS confirmation, DEG was spiked into a control cough syrup at 0.5% and analyzed on the LTQ-FT MS to serve as a positive control sample. The ammonium adduct of diethylene glycol was present at m/z 124.09654. The expected mass-to-charge ratio for this adduct is 124.09620, which is a difference of 2.25 ppm. MS–MS analysis revealed the presence of fragment ions at m/z 89.07 and 45.05. The fragment ion at m/z 89.07 corresponds to the structure CH2-CH2-O-CH2-CH2-OH, or a loss of water from diethylene glycol. The fragment ion at m/z 45.05 corresponds to the structure CH2-CH2-OH. A negative control consisted of unspiked cough syrup, and this control did not yield a peak at m/z 124.096.

Analysis of five of the experimental cough syrup samples yielded a precursor ion at approximately m/z 124.097, consistent with DEG. One of the red-colored antihistamine/expectorant samples from the implicated sample lot had a peak at m/z 124.09661, which corresponds to a difference of 1.69 ppm from the expected mass-to-charge ratio. The MS spectrum for this sample is shown in Figure 6. The MS–MS spectrum for this precursor ion is depicted in the inset of Figure 6. The fragment ions at m/z 89.07 and 45.05 verify that this compound is indeed DEG. All of the samples tested from the implicated product lot contained DEG. The observed mass-to-charge ratios for these samples corresponded to differences of less than 2 ppm in each case. The precursor ions in each case yielded fragment ions at
approximately $m/z$ 89.07 and 45.05, further verifying the presence of DEG in each of those samples. All other samples did not show the presence of characteristic DEG ions.

Library searches of the GC–TOF-MS and GC×GC–TOF-MS spectra of the implicated pharmaceutical lot found that DEG was present in each of the spectra with agreement of 84% or better than the reference spectrum for DEG. In addition, glycerin was found in all of the implicated samples indicating that DEG did not totally replace the glycerin diluent.

A mass chromatogram of the quantitative analysis of DEG in an implicated syrup, DEG, and control syrup are shown in Figure 7. The LOD of the quantitative method was approximately 1% with a relative standard deviation of about 10%. The relative recovery was equivalent to 100%. Concentrations of DEG measured in the implicated samples collected from patient families ranged from $8.1 \pm 1\%$ DEG (v/v). In samples with the same lot number collected from the manufacturer, the DEG content was $7.6 \pm 0.2\%$. Raw glycerin collected from the manufacturer contained $22.2 \pm 0.8\%$.

Similar to the Haiti incident, the pharmaceutical products were recalled, and the epidemic of acute renal failure ceased.

**General Discussion**

The continuing incidents of DEG mass poisoning has created the need for a standardized protocol for analyzing DEG and related glycols. As a result of the Panama epidemic, we have developed sensitive and selective methods to accurately quantify DEG in pharmaceutical products in the most timely manner possible. Reinventing detection and quantification methods for every incident requires a lot of work that is potentially unnecessary. However the constant evolution of the analytical equipment may necessitate the need to redevelop methods if years span between DEG mass poisonings. At CDC, we have decided to maintain a method that may be updated as instrumentation advancements occur so we will be best prepared if mass poisonings occur again.

Another way to expedite investigations of mass poisonings where pharmaceuticals are not clearly implicated or where the wrong pharmaceutical is linked is to develop biomarkers of exposure to DEG and related chemicals. To date, two methods have been reported in the literature that demonstrated the ability to quantify glycols enriched in urine or serum, but neither has shown that urinary DEG is a viable biomarker for DEG exposure. We have developed a method to measure urin-
nary DEG that should also be applicable to serum and other bodily fluids and will be reported separately. Our preliminary data indicate that we can significantly differentiate between those poisoned by DEG and those who were not. If we had this capability at the outset of the Panama investigation, we may have been able to implicate DEG as the etiologic agent allowing more immediate medical intervention despite the misleading link between lisinopril and the outbreak. Ultimately, however, we would have still needed to identify the source of exposure to control or cease the outbreak.

Conclusions

We have identified DEG as the contaminant in pharmaceutical elixirs in three mass poisoning epidemics of acute renal failure. For each contamination incident, different analytical techniques were used to ultimately identify DEG in the pharmaceutical products; however, each initial identification was made within 24 h, allowing a timely public health response. The continued occurrences of these epidemics had demonstrated a need to have standardized methodology available for an even more timely response, thus we have established such methodology. In addition, we have tentatively identified urinary DEG as a biomarker of DEG exposures capable of distinguishing those poisoned with DEG from those who did not ingest the poison.

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