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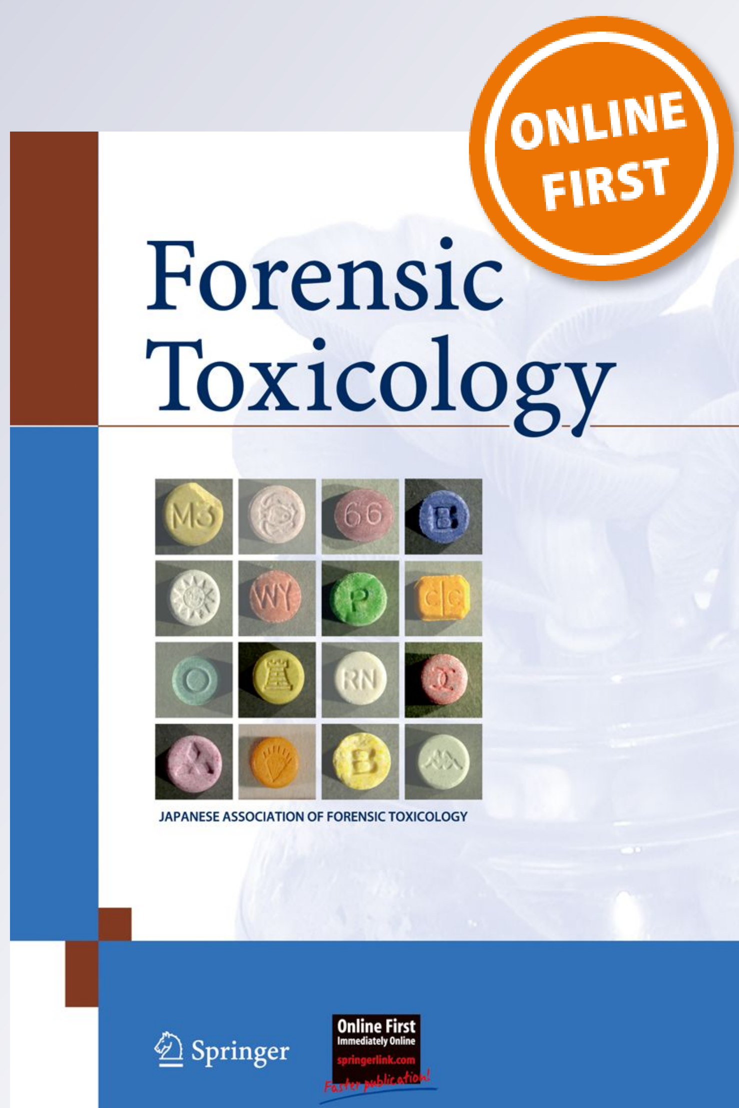

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LETTER TO THE EDITOR

Formation of semi-crystalline fraction, in which all diethylene glycol (DEG) is contained, during its extraction from human tissues: the probable cause of false negative results in fatal DEG poisoning cases

Leda Giannuzzi · Luis A. Ferrari

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Dear Editor,

Diethylene glycol (DEG) can be found in commercial products such as antifreeze, brake fluid, and lubricants. In addition, DEG has been found as a contaminant of raw materials in the production of pharmaceuticals. At least ten mass DEG poisoning events have occurred over the past 70 years. The first and largest outbreak, which resulted in 105 deaths, occurred in the United States in 1937 [1]. In 1967, a mass poisoning occurred in South Africa in which 7 children died [2]. In 1992 in Argentina, 29 people died after consuming propolis syrups that contained high DEG concentrations; the drug was widely commercialized in Argentina to treat mild upper respiratory tract infections [3]. Thereafter, pediatric medicinal syrups contaminated with DEG caused the deaths of 33 children in India in 1998 [4], and 85 children in Haiti in 1995–1996 [5]. The most recent outbreak took place in Panama in 2006, in which more than 100 people died due to DEG poisoning [1].

In spite of these repeated mass DEG poisonings, only a few analytical methods for DEG analysis, by gas chromatography (GC) [3] and GC–mass spectrometry (MS) [6–8], have been reported. There is a pressing need to establish and improve the methods for analysis of DEG, especially for postmortem human samples. As a result of our extensive experience in analysis of DEG in postmortem samples, we hereby report an important characteristic of DEG that we observed during sample extraction.

Common chemicals, including ethylene glycol, DEG, fatty acids, phospholipids, and cholesterols, were of the highest chromatographic purity commercially available. GC was performed on a Shimadzu GC-14 A equipped with a Shimadzu CR 4A integrator (Shimadzu, Kyoto, Japan). A J&W DB-Wax column (30 m × 0.53 mm i.d., 1.5 µm film thickness, Agilent, Santa Clara, CA, USA) was used. The injector temperature was set at 250 °C and the flame-ionization detector (FID) was set at 250 °C. An initial oven temperature of 110 °C was held for 2 min before a temperature ramp of 8 °C/min was used to reach the final temperature of 210 °C. The carrier gas was nitrogen (12 cm^3/min). For thin-layer chromatography (TLC) analysis of lipid components, an Iatroscan TLC–FID apparatus (Mitsubishi Kagaku, Iatron, Tokyo, Japan) was used .

Tissue samples (whole blood, liver, and kidney) were obtained from 15 victims of massive intoxication who ingested propolis syrups contaminated with DEG. Ten millilitres of blood was obtained by puncturing the femoral vein and was analyzed after 24 h. Fifty grams each of liver and kidney was taken from the victims and stored frozen at −20 °C until analysis (3 days after collection). We isolated DEG from tissues by continuous Soxhlet extraction with methanol for 12 h. The extract obtained from each sample was purified using a charcoal column and evaporated to 1 ml. Then 10 µl of the concentrated extract was used for analysis of DEG by GC–FID.

When the methanol extract was stored in a refrigerator at 2–4 °C for several hours, a semi-crystalline substance appeared, adhering to the glass wall of the test tube. When subjected to GC analysis, the supernatant fraction of the

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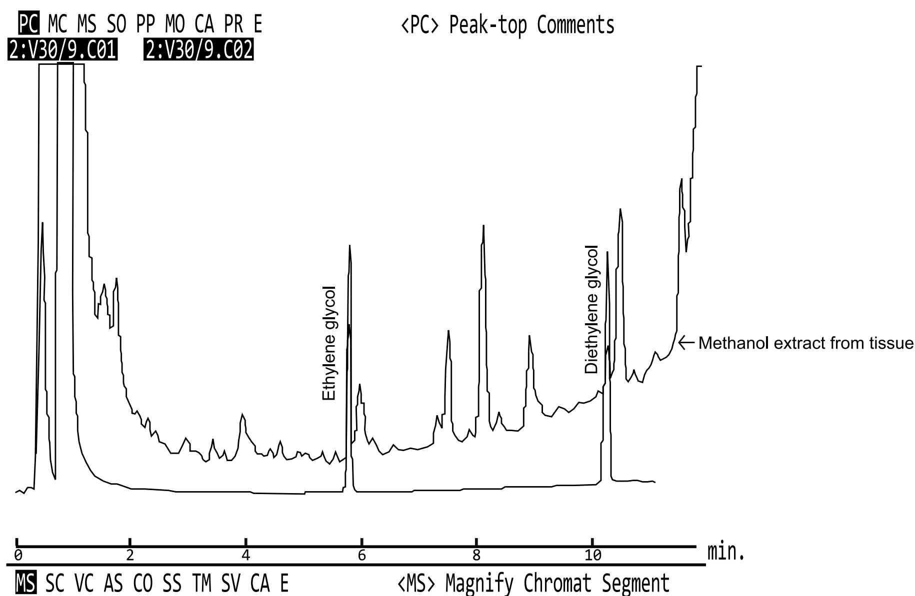

Fig. 1 Gas chromatography–flame ionization detection chromatogram for a methanolic extract obtained from a solid tissue of a victim poisoned by diethylene glycol. The sample was processed at room temperature (20 °C)

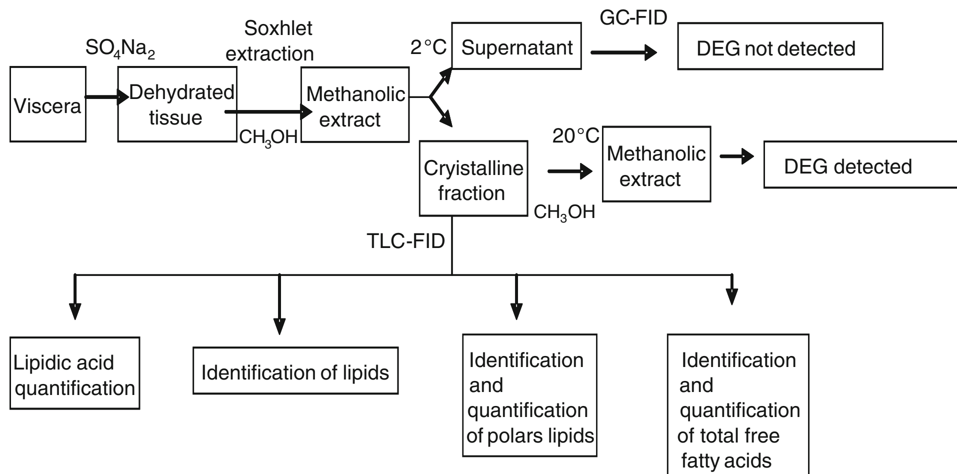

Fig. 2 Flowchart for extraction of diethylene glycol (DEG) from human whole blood, liver, or kidney and for analysis of lipid components

biphasic system gave negative results for DEG. In contrast, when the semi-crystalline substance was dissolved in a methanolic solution at room temperature for analysis, the GC–FID results became positive for DEG (Fig. 1), showing that most of the DEG was contained in the semi-crystalline substance.

Given observation of the above phenomenon, our attention was drawn to the composition of the semi-crystalline substance. The outline of the whole analytical procedure is shown in Fig. 2. The analysis of the substance was conducted by TLC–FID with toluene/chloroform/formic acid (75:25:2) or petroleum ether/toluene (70:30) as mobile phase with Silica Gel GF 254 (Merck, Darmstadt, Germany) as stationary phase. The composition of the semi-crystalline substances was 57.4 % free fatty acids (mostly palmitic, stearic, and oleic acids), 37.3 % phospholipids (phosphatidylethanolamine and sphingomyelin), and 5.3 % cholesterol. These results led us to reason that DEG forms a complex mainly with free fatty acids, probably by hydrogen and/or hydrophobic bonding, which results in the inclusion of DEG in the semi-crystalline substance at low temperature.

It is clear that literature reports of the detection of DEG in postmortem human materials are few in number [3], in spite of the huge number of deaths due to DEG poisoning over the past 70 years [1–5]. From our observations, we consider that the semi-crystalline substance, which forms during the extraction procedure at 0–5 °C and is removed by precipitation in most cases, contains almost all the DEG of the sample, and thereby causes false negative results for DEG. It is absolutely necessary to dissolve the semi-crystalline fraction in a solvent at room temperature before analysis if accurate results are to be obtained (Fig. 2).

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There are a few reports dealing with DEG analysis by GC [3] or GC–MS [6–8] for human specimens. In contrast to our report [3], others dealt with fresh human serum without cellular components including membranes [6–8]. While it appears much easier to extract DEG without the cellular components present, whole blood and/or solid tissues are frequently the matrices to be analyzed in postmortem toxicological practice, and these materials contain high amounts of phospholipids and fatty acids. In such cases, adequate treatment of the the semi-crystalline substance, as observed in our study, must be adopted to avoid false negative results for DEG.

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