Investigation of Fatalities Due to Acute Gasoline Poisoning

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Abstract

This paper presents a simple, rapid, reliable, and validated method suited for forensic examination of gasoline in biological samples. The proposed methodology has been applied to the investigation of four fatal cases due to gasoline poisoning that occurred in Spain in 2003 and 2004. Case histories and pathological and toxicological findings are described in order to illustrate the danger of gasoline exposure under several circumstances. Gasoline's tissular distribution, its quantitative toxicological significance, and the possible mechanisms leading to death are also discussed. The toxicological screening and quantitation of gasoline was performed by means of gas chromatography (GC) with flame-ionization detection, and confirmation was performed using GC–mass spectrometry in total ion chromatogram mode. m,p-Xylene peak was selected to estimate gasoline in all biological samples. Gasoline analytical methodology was validated at five concentration levels from 1 to 100 mg/L. The method provided extraction recoveries between 77.6% and 98.3%. The limit of detection was 0.3 mg/L, and the limit of quantitation was 1.0 mg/L. The linearity of the blood calibration curves was excellent with r² values of > 0.997. Intraday and interday precisions had a coefficient of variation ≤ 3.4% in all cases. Cases 1 and 2 consist of the accidental inhalation of gasoline vapor inside a small enclosed space. Case 3 is a death by recreational gasoline inhalation in a male adolescent. Heart blood concentrations were 28.4, 18.0, and 38.3 mg/L, respectively; liver concentrations were 41.4, 52.9, and 38.3 mg/kg, respectively; and lung concentrations were 5.6, 8.4, and 39.3 mg/kg, respectively. Case 4 was an accidental death due to gasoline ingestion of a woman with senile dementia. Peripheral blood concentration was 122.4 mg/L, the highest in our experience. Because pathological findings were consistent with other reports of gasoline intoxication and constituents of gasoline were found in the body, cause of death was attributed to acute gasoline intoxication. As a rule, this kind of poisoning offers little difficulty in diagnosis because there is a history of exposure, and the odor usually clings to the clothes, skin, or gastric contents. However, anatomic autopsy findings will be nonspecific and therefore toxicological analysis is necessary. There is a paucity of recent references regarding analytical and toxicological data, and this article provides evidence about toxic concentrations and is a useful adjunct to the postmortem toxicological interpretation of fatalities if the decedent has been involved in gasoline use.

Introduction

Gasoline (petrol, motor fuel, motor spirit, or benzin) is a highly flammable, mobile liquid with characteristic odor produced from the light distillates obtained during petroleum fractionation. The distillation from initial to final boiling point ranges from 32 to 225°C (90 to 437°F). Gasoline is widely used as fuel, solvent for rubber adhesives, extractant or diluent for essential oils, and finishing agent for artificial leathers. It consists of a mixture of C₄ to C₁₂ hydrocarbons. Natural gasoline, obtained by fractional distillation of petroleum, contains mostly saturated hydrocarbons, but the ordinary commercial grades of motor gasoline contain paraffins, olefins, napthenes (isoparaffins and cycloparaffins), and aromatics, all in substantial concentrations. Motor gasolines are made chiefly by cracking processes, in which heavier petroleum fractions are converted into more volatile fractions by thermal or catalytic decomposition (1–3).

In terms of chromatography, gasoline has a characteristic aromatic profile. The aromatic content of gasoline is similar to that of other petroleum distillates but more abundant than the aliphatic content. The aromatic profile of aromatic solvents may mimic gasoline, although with a narrower range with aliphatic compounds notably absent. The aliphatic profile of gasoline will vary by brand, grade, and lot, but it is always present. Most gasolines contain characteristic naphthalenes that may be absent in some northern winter markets (4). In accordance with European Standard, regular and premium unleaded petrol have a maximum limit of 42% of aromatics, 18–21% olefins, and 1% benzene (European Standard EN 228-January-2004, Automotive fuels-Unleaded petrol-Requirements and test methods, European Committee for Standardization).

Acute toxicity is similar for all petroleum distillates and consists of skin, eye, and respiratory tract irritation, CNS depression, and cardiac arrhythmias (5). In addition, gasoline can act...
as a simple asphyxiant if the vapors displace sufficient oxygen from the breathing atmosphere (6).

The number of exposures at which toxic effects occur is lower than exposures occurring through ingestion of contaminated water; inhalation of gasoline while filling the tank of cars; spending time at a petrol station; or employees working its production, distribution, and marketing (7). Compendia discuss gasoline toxicity largely in terms of anecdotal case reports (2,8–15), and only a few of them offer quantitative data (11,13,16–19).

The investigation of uncertain fatalities requires accurate determination of the cause of death, with assessment of all factors that may have contributed to it. In our institution, when solvents or petroleum distillates are suspected, a wider toxicological study of blood and tissues is added to the routinely toxicological investigations of alcohol (volatiles) and drugs. In this sense, for practical forensic medicine purposes, a reliable method for the detection and identification of small amounts of fuel components in body materials is required. This paper describes four fatal cases involving gasoline in Spain in 2003 and 2004 and illustrates the danger in several circumstances of gasoline exposure. The gasoline tissue distribution, quantitative toxicological significance, and the possible mechanisms leading to death are discussed along with a description of the validated analytical method.

Case Histories

Case 1
A 36-year-old miner was found dead after inhaling the combustion fumes of a broken motor inside a 5-m well. Inquiries indicated that the man had fallen into a well containing water; inhalation of gasoline while filling the tank of cars; spending time at a petrol station; or employees working its production, distribution, and marketing (7). Compendia discuss gasoline toxicity largely in terms of anecdotal case reports (2,8–15), and only a few of them offer quantitative data (11,13,16–19).

The investigation of uncertain fatalities requires accurate determination of the cause of death, with assessment of all factors that may have contributed to it. In our institution, when solvents or petroleum distillates are suspected, a wider toxicological study of blood and tissues is added to the routinely toxicological investigations of alcohol (volatiles) and drugs. In this sense, for practical forensic medicine purposes, a reliable method for the detection and identification of small amounts of fuel components in body materials is required. This paper describes four fatal cases involving gasoline in Spain in 2003 and 2004 and illustrates the danger in several circumstances of gasoline exposure. The gasoline tissue distribution, quantitative toxicological significance, and the possible mechanisms leading to death are discussed along with a description of the validated analytical method.

Case 2
A 26-year-old employee of a gas station suffered cardiorespiratory insufficiency after accidental inhalation of gasoline. He was discovered in the service station unconscious with weak radial and femoral pulses. He had been repairing a 160 × 60-cm box with tubes conducting to a gasoline tank. He suffered several cardiac arrests while being transported to the hospital and in spite of cardiorespiratory rescue. He was pronounced dead on arriving at the hospital.

Necropsic findings included extensive blistering and peeling of skin over the left part of the trunk, right upper arm, and thigh, which is consistent with burns from gasoline. Bilateral pulmonary edema and generalized visceral congestion were other findings. A semiliquid content of blackish discoloration was found in stomach.

Peripheral and heart blood, urine, vitreous humor, liver, kidney, lung, brain, and gastric content (total amount: 110 mL) were sent for analysis.

Case 3
A 15-year-old male was found in cardiac arrest at home. Inquiry suggested the hypothesis that the boy had intentionally inhaled gasoline, glue, and perhaps other solvents. Near the body, several chemicals as pure substances or in domestic and industrial mixtures such as gasoline, glue, turpentine, and paints were found. Neither lesions nor wounds were found during corpse examination. He also abused hashish.

Peripheral and heart blood, bile, vitreous humor, liver, kidney, lung, and gastric content (total amount: 250 mL) were collected at autopsy as well as the product (glue) present in a plastic bag.

Case 4
A 73-year-old female with senile dementia was found 10 h after her death. A liquid smelling strongly of petroleum distillates was expelled from her mouth. No pathological findings were provided by the medical examiner. The etiology was considered accidental ingestion and the immediate cause of death, multiorganic failure. Peripheral blood and vitreous humor were collected and sent for analysis.

Experimental

Materials
Sodium sulfate, diethyl ether, and methanol of analytical grade were obtained from Scharlau (Barcelona, Spain). Unleaded gasoline neat standard was purchased from NSI Solutions, Inc. (Raleigh, NC), gasoline constituents were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and n-octylbenzene (internal standard, IS) was purchased from Fluka-Sigma Aldrich (Buch, Switzerland). The density of the gasoline standard, 0.75 g/mL, was used as a multiplication factor in order to convert microliters to milligrams. Stock solutions of gasoline (1 and 10 mg/mL) and IS (1 mg/mL) were prepared by dissolving the appropriate amount in methanol. These stock solutions were stored in glass tubes and maintained at −25 °C until used. These solutions were used to prepare blood calibration standards of 1, 5, 10, 50, and 100 mg/L by adding the appropriate amounts of gasoline to a pool of citrated human whole blood samples provided by Comunidad de Madrid Blood Bank (Madrid, Spain) and previously verified the possibility for use as blanks. No interferences were found for the studied compounds, and the samples were kept frozen at −25 °C until used.

Specimens
Specimens were collected at autopsy, preserved, and frozen
until analysis. Tissues were homogenized using a mixer-blender. Specimens in which the amount of gasoline exceeded the linear range of the assay were diluted by an additional factor of 10 prior to re-extraction.

Extractions

All biological samples, including blanks, controls, and standards, were processed according to the described one-step liquid–liquid extraction procedure. A 3-mL aliquot of each liquid sample (3-g of each tissue) was transferred to a 10-mL screw-capped glass tube; combined with 100 μL IS solution (n-octylbenzene methanolic solution of 100 mg/L), 1 mL of diethyl ether (cold at 4°C), and 15 mg of anhydrous sodium sulfate; vortex mixed for 3 min; and centrifuged at 4000 rpm for 10 min (at 4°C). Then the organic layer was collected and transferred to a gas vial, and 3 μL was injected first for gas chromatography–flame-ionization detection (GC–FID) screening analysis and quantitation and then GC–mass spectrometry (MS) confirmation of the obtained results.

Apparatus

GC–FID analysis was performed with an HP 5890 series II apparatus, provided with an HP 7673A autosampler, linked to an HP 3396A integrator with a 25-m (0.20-mm i.d., 0.11-μm film thickness) Ultra-1 HP cross-linked methylsilicone column (Hewlett-Packard, Avondale, PA). The GC conditions were as follows: helium carrier gas (Air Liquid, Madrid, Spain) at a column head pressure of 22 psi, split mode (split ratio 1:24), injector temperature 280°C, oven temperature programmed from 40°C (initial time 3 min) to 280°C at 10°C/min, and detector temperature 300°C. The total chromatographic time, including 2 min of equilibration time, was 29 min. The detector gases were hydrogen and air (Air Liquid), delivered at a flow rates of 40 and 400 mL/min, respectively. Insert liners silanized with dimethylchlorosilane/toluene (5:100) and packed with Supelco silanized glass wool (Supelco Park, Bellefonte, PA) were used.

GC–MS analysis was performed with an HP 5971A mass-selective detector controlled by an HP 1034 C ChemStation (Hewlett-Packard). The autosampler, GC, and column were as mentioned. The MS conditions were as follows: Total ion chromatogram (TIC) mode (m/z 35–650), electron impact ionization with a 70 eV energy, and transfer line and ion-source temperatures were both maintained at 280°C. The calibration curves were generated from least-squares linear regression. The regression lines were used to calculate the absolute recoveries (n = 6) of gasoline from spiked blood at five concentration levels.

The intraday precision was assessed at five concentration levels by the extraction and analysis on the same day of six spiked blood samples for each level.

The interday precision was assessed by analyzing a set of ten spiked blood samples at five concentrations on different days, by different operators, using fresh gasoline in diethyl ether calibration curves.

The limits of detection (LOD) and quantitation (LOQ) were determined as the lowest concentration giving a response of 3 and 10 times, respectively, the average of the base line noise defined from six control samples.

The linearity of the method were checked by preparing six replicates of the calibration curves at five different concentrations, ranging from 1 to 100 mg/L, by addition of known amounts of gasoline to human blood.

Drug screens and other analysis

Blood/urine from each case was screened by immunonassay for propoxyphene, cocaine and benzoylcegonine, methadone, opiates, cannabinoids, benzodiazepines, amphetamine (and related compounds), barbiturates, and tricyclic antidepressants on a Hitachi 902 Automatic Analyzer (Tokyo, Japan) using Cedila® reagents (Microgenics, Fremont, CA). In addition, blood and urine, when available, from each case were extracted with Bond-Elut Certify columns collecting together the acidic-neutral and basic eluates. Each sample extract was analyzed by GC with nitrogen-phosphorus detection for screening analysis, by GC–MS for confirmation of the results, and high-performance liquid chromatography–scanning UV detection. Blood and urine from each case were also examined for ethanol and other volatiles (methanol, acetone, n-propanol, and isopropanol) using headspace with GC–FID. In cases 1–3, the percentage of carboxyhemoglobin was also determined.

Results and Discussion

Assay characteristics

There is a lack of data in the literature about methods to measure tissue concentrations in gasoline deaths, and information about tissue concentrations and distribution studies is very limited (13,17,21–25). Although gasoline is a "complex hydrocarbon mixture", it is a product and requires testing like any other product, taking into account that it can be involved in forensic cases. Therefore, for practical forensic medicine purposes, a simple and reliable analytical method for application in cases of gasoline poisoning in body materials is necessary.

Gasoline has a characteristic aromatic chromatographic profile. The GC–FID patterns of gasoline, diesel-fuel, kerosene,
turpentine, etc., cited as some of the most representative examples of flammable or combustible liquids that can be involved in poisoning cases (as well as at the scene of a suspected arson), are very different from each other. Figure 1 shows comparative chromatograms of these ignitable liquids under the described chromatographic conditions in which major peak identification is provided. Additionally, an identification list of standard gasoline components under the described chromatographic conditions is showed in Table I.

The proposed analytical method allows a comprehensive toxicological screening for solvents and other petroleum distillates in which gasoline is included. The method is based on a previously developed method at National Institute of Toxicology and Forensic Sciences (Madrid, Spain) for capturing ignitable liquids (accelerants) from fire debris, from fragments of clothes, and/or skin obtained from burnt bodies in homicide cases (unpublished). In all these cases the chromatogram of a questioned petroleum product is compared with a library of chromatograms of distillates. The screening method is based in the pattern recognition methods widely applied in many areas of forensic science.

In the four presented cases, gasoline components were easily identified by comparing chromatography of the gasoline standard to that of the samples using the GD–FID screening method. Figure 2 shows representative GC–FID chromatograms of gasoline standard, blank blood, gasoline-spiked blood, and blood and tissues obtained from the forensic cases. On close inspection, both the standards and biological samples exhibited chromatograms consistent in the region from 2.0 to 12.5 min but there were differences in some relative peaks ratios. This can be justified by differences in route of exposure, rate of metabolism, and abundance of gasoline components. Additionally, the possible creation of new species in the pathway to elimination is another important consideration when comparing these chromatograms. Furthermore there are variations due to the different capacities of the hydrocarbons for diffusion through the cell wall, correlating with the different liposolubilities of each constituent and the accumulation in different organs (16,26). These facts complicate the estimation of gasoline concentration because the values calculated depend on the hydrocarbon taken as a reference.

Different authors selected different peaks detected by GC–FID to estimate gasoline in blood and tissues. Nagata and Fujiwara (16) selected two peaks, corresponding to two unidentified gasoline constituents, to estimate gasoline in blood. Nelms et al. (13) reported a fatality due to inhalation of aviation gasoline, which differs from automotive gasoline in composition, and selected another certain constituent for quantitation. Carnevale et al. (17) reported that the relative amounts of single hydrocarbons were extremely variable depending on the sample examined, and because 2-methylpentane was present in all the organs in a constant ratio, it was used to estimate gasoline concentration.
concentrations in biological samples. Kimura et al. (18) calculated concentrations with benzene and Takamiya et al. (23) chose toluene. Nagata et al. (16) and Byard et al. (19) used benzene, toluene, o,m,xylene, and propylenebenzene to estimate gasoline concentrations in biological samples.

Among constituents of gasoline, toluene, xylene, and trimethylbenzenes proved ubiquitous in the four cases as major gasoline concentrations in biological samples. We chose to use the xylene component always present in our chromatograms. Furthermore, its volatility is intermediate—between toluene and trimethylbenzenes—and thus there is less risk of losses due to storage or handling during extraction, but it is still more abundant than trimethylbenzenes, which are less volatile. The selection of toluene as reference peak for quantitation was discarded because in case 3, the young victim abused both substances, toluene (glue) and gasoline.

Although no doubt was maintained regarding the identity of the source of poisoning after analyzing all samples on the GC-FID, a decision was made to re-analyze certain samples of each case on the GC-MS for specific peak confirmation. As a representative example, Figure 3 shows a zoom of the TIC and mass ions obtained from the heart blood sample of case 4 by GC-MS under the described chromatographic conditions.

Quantitative analysis was undertaken by GD-FID using blood calibration curves in the range of 1–100 mg/L and using m,p-xylene peak for all gasoline calculations. Additionally, in each batch an extracted gasoline-spiked blood sample (25 mg/L) and a gasoline standard in diethyl ether (75 mg/L), both prepared from a different stock solution of gasoline, were assayed as controls. Toxicological findings for the four medical examiner cases are shown in Table II. Regarding alcohol and other volatiles, abused and therapeutic drugs screens, the results were negative in all the cases.

A summary of the validation data of this analytical method are shown in Table III. The method is simple, selective, precise, sensitive, and reliable for gasoline detection in forensic cases. Good linearity and excellent recoveries were also obtained.

For toxicological screening in our method, n-octylbenzene has been employed as an IS because it is absent in gasoline and other petroleum distillates and commercial products, as far as we know. Its chemical structure makes it suitable for use as an internal standard for a wide variety of compounds in the performance of a general screening procedure. According to our toxicological analytical experience it has a very good extraction behavior (the recovery obtained for this standard was 103% with a relative standard deviation of 5%).

### Poisoning characteristics

Gasoline is mainly used for internal combustion engines, but its universal availability encourages misuse in the home as a solvent, cleaning solution, or abused substance. A number of non-lethal acute cases of gasoline intoxications are registered annually by poison centers [data from our Poison Control Center (unpublished), (15)], usually the result of the siphoning of petrol for fuel tanks or children drinking from contaminated storage containers. Other cases include fire-eaters who accidentally aspirated petroleum during the flame-blowing act (27).

We report here four lethal cases of gasoline exposure under different circumstances. In all of them, gasoline components were identified and quantified by the use of m,p-xylene peak in our standard. No underlying organic diseases were found and toxicological evaluation of blood did not reveal alcohol or any common drugs that could have caused or contributed to death. The major route of occupational exposure to gasoline is inhalation. Cases 1 and 2 consist of the accidental inhalation of gasoline vapor inside a small enclosed space. Fatal occupational exposure reports have included workmen cleaning storage tanks or people entering a petrol tank without a mask respirator to rescue them (12,23). Other accidental lethal cases in cars are reported in the literature (11,13,19,21,22). Gasoline vapors at 2000 ppm produce mild anesthesia in 30 min

### Table I. Identification Table of Gasoline Components by Their GC Retention Time and Mass Spectra Under the Described Chromatographic Conditions

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>ART</th>
<th>RRT</th>
<th>Ions m/z (inten.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benene</td>
<td>78.11</td>
<td>2.01</td>
<td>0.06</td>
<td>78100</td>
</tr>
<tr>
<td>Iso-octane</td>
<td>114.21</td>
<td>2.30</td>
<td>0.08</td>
<td>99, 5710041127</td>
</tr>
<tr>
<td>n-Heptane</td>
<td>100.20</td>
<td>2.41</td>
<td>0.09</td>
<td>10035, 6151712257243100</td>
</tr>
<tr>
<td>Toluene</td>
<td>92.13</td>
<td>3.07</td>
<td>0.13</td>
<td>9259, 91100651251396</td>
</tr>
<tr>
<td>n-Octane</td>
<td>114.23</td>
<td>3.90</td>
<td>0.19</td>
<td>11415, 85100713957243100</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>106.16</td>
<td>4.79</td>
<td>0.26</td>
<td>10652, 911007712656518</td>
</tr>
<tr>
<td>m-Xylene</td>
<td>106.16</td>
<td>4.97</td>
<td>0.27</td>
<td>10652, 911007712656518</td>
</tr>
<tr>
<td>p-Xylene</td>
<td>116.16</td>
<td>4.97</td>
<td>0.27</td>
<td>11652, 911007712656518</td>
</tr>
<tr>
<td>o-Xylene</td>
<td>116.16</td>
<td>5.41</td>
<td>0.30</td>
<td>11652, 911007712656518</td>
</tr>
<tr>
<td>n-Nonane</td>
<td>127.99</td>
<td>5.95</td>
<td>0.34</td>
<td>12799, 12863, 71257100</td>
</tr>
<tr>
<td>n-Propylbenzene</td>
<td>120.09</td>
<td>6.70</td>
<td>0.39</td>
<td>12025, 911006510</td>
</tr>
<tr>
<td>3-Ethyltoluene</td>
<td>120.20</td>
<td>6.87</td>
<td>0.41</td>
<td>12034, 105109110</td>
</tr>
<tr>
<td>4-Ethyltoluene</td>
<td>120.20</td>
<td>6.90</td>
<td>0.41</td>
<td>12034, 105109110</td>
</tr>
<tr>
<td>1,3,5-Trimethylbenzene</td>
<td>120.20</td>
<td>7.02</td>
<td>0.42</td>
<td>12034, 1051091109712</td>
</tr>
<tr>
<td>2-Ethyltoluene</td>
<td>120.20</td>
<td>7.19</td>
<td>0.43</td>
<td>12034, 1051091109712</td>
</tr>
<tr>
<td>1,2,4-Trimethylbenzene</td>
<td>120.20</td>
<td>7.49</td>
<td>0.45</td>
<td>12034, 1051091107712</td>
</tr>
<tr>
<td>1,2,3-Trimethylbenzene</td>
<td>120.20</td>
<td>8.00</td>
<td>0.49</td>
<td>12034, 1051091107712</td>
</tr>
<tr>
<td>1,2,4,5-Tetramethylbenzene</td>
<td>134.22</td>
<td>9.77</td>
<td>0.62</td>
<td>11910, 10591614</td>
</tr>
<tr>
<td>1,2,3,5-Tetramethylbenzene</td>
<td>134.22</td>
<td>9.82</td>
<td>0.62</td>
<td>11910, 10591614</td>
</tr>
<tr>
<td>1,2,3,4-Tetramethylbenzene</td>
<td>134.22</td>
<td>10.34</td>
<td>0.66</td>
<td>11910, 10591614</td>
</tr>
<tr>
<td>Naphtalene</td>
<td>128.06</td>
<td>10.68</td>
<td>0.68</td>
<td>12890, 1286351</td>
</tr>
<tr>
<td>1-Methylnaphtalene</td>
<td>142.08</td>
<td>12.45</td>
<td>0.81</td>
<td>14225, 11510715</td>
</tr>
<tr>
<td>n-Octylbenzene (IS)*</td>
<td>190.17</td>
<td>15.13</td>
<td>1.00</td>
<td>19032, 133492100</td>
</tr>
<tr>
<td>Ionol (ethyl ether stablizer)</td>
<td>220.18</td>
<td>15.62</td>
<td>1.04</td>
<td>22032, 2051001777</td>
</tr>
</tbody>
</table>

* Abbreviations: MW, molecular weight; ART, absolute retention time (min); RRT, relative retention time to n-octylbenzene (IS).
* These chemical products are not gasoline components.
Figure 2. Representative GC-FID chromatograms of the medical examiner cases: gasoline standard (75 mg/L) (A); blank blood (B); gasoline spiked blood (25 mg/L) (C); heart blood (D) and liver (E) obtained from case 1; brain obtained from case 2 (F); liver obtained from case 3 (G); and peripheral blood obtained from case 4 (H). Peak identification: 1, isooctane; 2, toluene; 3, ethylbenzene; 4, m,p-xylene; 5, o-xylene; 6, 3-ethyltoluene; 7, 1,2,4-trimethylbenzene; 8, 1,2,3-trimethylbenzene; 9, 1,2,4,5-tetramethylbenzene; 10, naphtalene; 11, 1-methylnaphtalene; 12, n-octylbenzene (IS); 13, ionol (diethyl ether stabilizer); and 14, fatty acids.
Short-term exposure to more than 5000 ppm would be lethal in less than 5 min of exposure (12,16,25). Gasoline is frequently handled where internal combustion engines are operating, and exhaust fumes also contain carbon monoxide (28). The color of the mucous membranes and the percentage of carboxyhemoglobin serves to identify the latter condition. In case 1, death occurred after the inhalation of exhaust fumes with several noxious substances including low molecular weight hydrocarbons and carbon monoxide.

Case 3 is a death by recreational gasoline inhalation. Toluene was one of the first substances recognized as giving rise to glue-sniffing. Gasoline is the second of substances of deliberate inhalational abuse, especially among male adolescents in lower-income populations (29), primarily those in rural areas or aboriginal people (30-33) because of the ready availability and relatively low cost (10,11,25,34-36). The ratio of deaths is relatively small, and about 70% of deaths occur under the age of 20.

Several cases of death due to hydrocarbons have been reported as having been caused by gasoline ingestion (17,37), as in our case 4. As little as 10 to 15 g may be fatal in children, and 20 to 50 g can cause severe intoxication in adults (8,38). On the other hand, smaller amounts may lead to a fatal outcome due to pulmonary complications (7).

Gasoline deaths are attributed to the lowering of the myocardial threshold to the arrhythmogenic action of epinephrine (38,39), or to CNS depression and respiratory failure (40). Besides, effects on the CNS such as incoordination, confusion, and loss of consciousness can explain accidents, such as drowning as described in case 1. Furthermore, the early CNS effects may lead to behavioral aberrations which diminish the victim's ability to rescue himself.

Postmortem examination usually reveals little (10), except acute lung congestion and in volatile inhalant abuse, burns to the mouth (32). Common findings are chemical pneumonitis, atelectasis, and bronchopneumonia, but if death occurs within a few hours as in the presented cases, few changes may be seen other than edema and congestion or scattered hemorrhages in the lungs (9). Chemical dermal burns are due to prolonged contact of the skin with petrol (5,11,19,41,42) as described in case 2. In addition, victims with marked gasoline burns, suggests that significant transcutaneous absorption of hydrocarbons might have also resulted from skin exposure (32).

Toxicological analysis was performed in order to verify gasoline exposure and to study distribution in the body. Gasoline is absorbed slowly from the stomach after ingestion (8), although in respiratory exposure gasoline could be detected in stomach and bile. Excretion is primarily by the lungs and kidneys.

Highest concentrations of gasoline (measured as m,p-xylene) were found in blood after oral ingestion. In other publications, blood lethal concentrations ranged between 52 and 247 mg/L in oral and respiratory exposures, respectively (17,22). Differences could be due to the circumstances around the death such as co-intoxication with carbon monoxide or toluene. Besides, the half-life for hydrocarbons is short so the concentration at the time of the accident must have been considerably higher. False quantitation in volatile substance abuse could result from losses of analytes during sampling and vigorous resuscitation will blow off much of this solvent in the lungs (43). As mentioned previously, another problem is that there is not a unique criterion for the selection of peaks for quantitation.

Case 2 and 3, in which a more complete study was carried out of gasoline distribution, liver, lung, and brain concentrations were the highest, especially in case 3 of recreational gasoline sniffing. Only one other author has quantified concentrations in a human poisoning in different tissues after inhalation. However, the inhaled product was aviation gasoline (13). In a case of fatal poisoning by ingestion reported by Carnevale et al. (17), distribution of gasoline showed the highest concentrations in the lung, liver, and brain. Low concentrations in vitreous humor indicate that lipophylic substances such as hydrocarbons do not concentrate in this biological fluid. Toluene in case 3, was detected at 12.5 mg/L in heart blood. Baselt (3) quotes a fatal range of toluene blood concentrations between 10 and 20 mg/L with an average of 13 mg/L for fatalities. This showed that toluene could have contributed to death. Nevertheless, toluene is also a constituent of gasoline, and the source of this solvent in case 3 could have been both gasoline and the glue.

**Conclusions**

As conclusion, the proposed validated analytical method is simple, rapid, precise, reliable, and suited to implementation in...
the forensic toxicological examination of biological samples for gasoline. In addition, an account is given of the postmortem findings in four decedents from gasoline. As a rule, acute petroleum distillates poisoning offers little difficulty in diagnosis. There is a history of exposure and the odor usually clings to the clothes, skin, or gastric contents. However, anatomic autopsy findings will typically be nonspecific and therefore toxicological analysis is necessary. As there is a paucity of recent references, this article provides new data about toxic concentrations and is a useful adjunct to the postmortem toxicological interpretation of fatalities if the decedent has been involved in gasoline use. In addition, with complex compounds such as petroleum distillates, the development of standardized methodology in order to be able to compare data from different authors in different laboratories is urgently needed.

Table II. The Gasoline Concentrations in Various Body Fluids and Tissues from Four Medical Examiner Cases*

<table>
<thead>
<tr>
<th>Cause/Manner of Death</th>
<th>Case 1 Acute gasoline inhalation/accidental</th>
<th>Case 2 Acute gasoline inhalation/accidental</th>
<th>Case 3* Acute gasoline + toluene inhalation/abuse</th>
<th>Case 4 Acute gasoline ingestion/accidental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>NA*</td>
<td>19.3</td>
<td>22.1</td>
<td>122.4</td>
</tr>
<tr>
<td>Heart blood (mg/L)</td>
<td>28.4</td>
<td>18.0</td>
<td>38.3</td>
<td>NA</td>
</tr>
<tr>
<td>Heart blood (%COHb)</td>
<td>%COHb = 9%</td>
<td>%COHb = 1%</td>
<td>%COHb = 6%</td>
<td></td>
</tr>
<tr>
<td>Urine (mg/L)</td>
<td>NA</td>
<td>2.3</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Bile (mg/L)</td>
<td>NA</td>
<td>NA</td>
<td>16.9</td>
<td>NA</td>
</tr>
<tr>
<td>Vitreous (mg/L)</td>
<td>NA</td>
<td>NA</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Liver (mg/kg)</td>
<td>41.4</td>
<td>52.9</td>
<td>124.2</td>
<td>NA</td>
</tr>
<tr>
<td>Kidney (mg/kg)</td>
<td>NA</td>
<td>34.9</td>
<td>43.1</td>
<td>NA</td>
</tr>
<tr>
<td>Lung (mg/kg)</td>
<td>5.6</td>
<td>8.4</td>
<td>39.3</td>
<td>NA</td>
</tr>
<tr>
<td>Brain (mg/kg)</td>
<td>NA</td>
<td>65.6</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Stomach (mg/L)</td>
<td>1.1</td>
<td>65.8</td>
<td>19.4</td>
<td>NA</td>
</tr>
<tr>
<td>Total amount (mL)</td>
<td>0.7</td>
<td>7.3</td>
<td>6.5</td>
<td></td>
</tr>
</tbody>
</table>

* m,p-Xylene peak was the reference peak for all gasoline calculations.
* Case 3: The content of the plastic bag found near the corpse was identified as toluene (glue).
* Abbreviations: NA, not available; T, Toluene; % COHb, % of carboxyhemoglobin.
* Total amount of gasoline (mL)/total amount of gastric content.

Table III. Validation Data of Gasoline from Fortified Whole Blood Samples Using Liquid–Liquid Extraction and Analyzed by GC-FID

<table>
<thead>
<tr>
<th>Gasoline Concentration (mg/L)</th>
<th>% Recovery (n = 6)</th>
<th>Intraday Precision (RSD%) (n = 6)</th>
<th>Interday Precision (RSD%) (n = 10)</th>
<th>Linearity (r²) (n = 6)</th>
<th>LOD (mg/L)</th>
<th>LOQ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>77.6</td>
<td>2.0</td>
<td>4.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>86.7</td>
<td>3.8</td>
<td>4.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>89.4</td>
<td>3.9</td>
<td>4.5</td>
<td>0.997</td>
<td>0.3</td>
<td>1.0</td>
</tr>
<tr>
<td>50</td>
<td>86.3</td>
<td>3.0</td>
<td>4.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>98.3</td>
<td>3.5</td>
<td>5.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* RSD, relative standard deviation.

References

6. ACGIH: 2003 Threshold Limit Values (TLVs(R)) for Chemical Substances and Physical Agents and Biological Exposure Indices (BEIs(R)), American Conference of Governmental Industrial Hygienists, Cincinnati, OH, 2003.


