Suicidal Inhalation of Motorbike Exhaust: Adding New Data to the Literature about the Contribution of Gasoline in the Cause of Death

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Abstract

We would like to alert toxicologists to the importance of testing for gasoline, and for volatile hydrocarbons in general, in deaths involving inhalation of exhaust fumes occurring in closed spaces with running motors or machinery. We present here a case of suicidal inhalation of motorbike exhaust, a mixture of carbon monoxide (CO) and gasoline vapor, by a 38-year-old female. She was found in her closed home garage with a hose extending from the exhaust pipe of a motorbike through a cellophane plastic device into a closed tent in which the victim lay. She left two suicide notes nearby. The carboxyhemoglobin (COHb) was measured using visible spectrophotometry. The toxicological screening and quantification of gasoline was performed by means of gas chromatography with flame-ionization detector and confirmation was performed using gas chromatography–mass spectrometry. The %COHb determined in blood was 73%. Gasoline concentrations in heart blood and vitreous humor were 22.3 and 1.0 mg/L, respectively. Although fatalities with CO at this rate are common, we would like to highlight the role of gasoline and add new quantitative data of this toxic substance to the scarce literature. Based upon the toxicological data, along with the information provided by the medical examiner, the cause of death was determined to be CO and gasoline poisoning and the manner of death suicide.

Introduction

Fuel-powered equipment is commonly used in industrial and household operations. Fuel emissions include oxides of nitrogen, carbon, sulfur, respirable particulate matter, and unburned hydrocarbons, among others. Combustion mixtures that burn an inefficiently high concentration of fuel produce more hydrocarbons, particulate matter, and carbon monoxide (CO). In an experimental study, Massad et al. (1) concluded that gasoline exhaust fumes contain noxious substances such as volatile hydrocarbons which besides CO, are responsible for increasing toxicity. Conditions where these incidents occurred included suicides and accidental cases due to poor engine maintenance, prolonged idling of machinery, use of equipment during times when ventilation was disrupted or use of poor-quality fuel (2).

CO is impossible to detect by an exposed person because it is a colorless, tasteless, odorless, and non-irritating gas. Exposure to 0.4% (v/v) CO in air can be fatal in less than 1 h. The first accurate description of CO poisoning was recorded by Claude Bernard in 1857. Since then, many advances in our understanding of the pathophysiology have been achieved. CO toxicity results from a combination of tissue hypoxia and direct mediated damage at the cellular level. When inhaled, CO is readily absorbed from the lungs into the bloodstream where it forms a complex with hemoglobin known as carboxyhemoglobin (COHb). The presence of COHb decreases the oxygen carrying capacity, reducing the availability of oxygen, and resulting in tissue hypoxia. The presence of COHb increases the stability of the remaining oxyhemoglobin and shifts the oxygen dissociation curve to the left, so less oxygen is released.

Gasoline is a highly flammable liquid with characteristic odor produced from the light distillates obtained during petroleum fractionation. Gasoline is widely used as a 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. In accordance with the European Standard, regular and premium unleaded petrol have a maximum limit of 42% of aromatics, olefins 18–21%, and benzene 1% (European Standard EN 228-January-2004, Automotive fuels-Unleaded petrol-Requirements and test methods. European Committee for Standardization). In terms of chromatography, gasoline has a characteristic aromatic profile.

Gasoline can lead to cardiac arrhythmias due to the sensitization of the myocardium to catecholamines (3–5). Besides, gasoline can act as a simple asphyxiant if the vapors displace sufficient oxygen from the breathing atmosphere (6). Inhaled gasoline is absorbed faster than ingested gasoline and produces toxicity (7–13).

The investigation of uncertain fatalities requires accurate...
Case History

In January 2006, a 38-year-old female was found dead after inhaling the combustion fumes of a motorbike inside the closed garage of her house. She was found supine with signs of therapeutic interventions applied by the emergency health staff.

In the garage, near the victim there was a motorbike, with a running two stroke motor, and with the exhaust pipe connected with a hose. In the other side of the hose there was a plastic device connected with adhesive cellophane to a tent and inside the tent lied the victim. In the house there were two manuscript notes written by the victim.

Body examination revealed a scar of thoracotomy, rigor mortis compatible with the data of death, and cherry red lividity in decline zones. The remainder of the external examination was unremarkable. Internal examination showed cherry colored lungs and signs of surgery in the left atrial-ventricular valve. The right atrial-ventricular valve had thick valves and adherences. Other significant findings were hypertrophy of the left ventricle and diffuse congestion of viscera.

Neither bone fractures nor visceral injuries were observed. There was no known drug-abuse history or a present natural disease to account for the death. The patient had had a cardiac disease with surgical intervention of the left atrial-ventricular valve.

Heart blood and vitreous humor were sent for analysis to our laboratory.

Experimental

Toxicological analysis

A full toxicological analysis was performed on the deceased's heart blood. The COHb was determined in blood using an spectrophotometric procedure previously published by Rodkey et al. (14). The absorption of the blood sample was determined following its dilution (1000-fold) with sodium dithionite and ammonium hydroxide. The diluent providing the two components COHb-Hb for absorbance measurements at 420 and 432 nm using a Perkin Elmer 550 UV-vis spectrophotometer (Perkin Elmer, Boston, MA). Blood was also examined for ethanol and other volatiles (methanol, acetone, and isopropanol) using headspace with gas chromatography with flame-ionization detector (GC-FID). Additionally, blood from the case was screened by immunoassay for propoxyphene, cocaine and benzoylcegonine, methadone, opiates, cannabinoids, benzodiazipines, amphetamine (and related compounds), barbiturates, and tricyclic antidepressants on a Hitachi 902 Automatic Analyzer (Tokyo, Japan) using Cedix® reagents (Microgenics, Fremont, CA). Then, the blood sample was extracted with Bond-Elut Certify columns (Varian Sample Preparation Products, Harbor City, CA) collecting together the acidic-neutral and basic eluates. The sample extract was analyzed by GC–nitrogen-phosphorus detector (NPD) for screening analysis and by GC–mass spectrometry (MS) for confirmation analysis following an analytical method described in our previous work (15). Finally, the heart blood sample was analyzed for petroleum distillates/hydrocarbons following our previously described method (16).

Quantitation of gasoline

Materials and reagents. Sodium sulfate, diethyl ether, and methanol of analytical grade were obtained from Scharlau (Barcelona, Spain). Unleaded gasoline neat standard was purchased from NSI Solutions (Raleigh, NC), 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 milliliters to milligrams. Stock solutions of gasoline (1 and 10 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 to prepare blood calibration standards by spiking the appropriate amounts of gasoline to negative blood samples obtained from Comunidad de Madrid Blood Bank (Madrid, Spain).

Sample preparation. Specimens collected at autopsy were preserved and frozen until analysis. Heart blood, vitreous humor, blank blood, control, and standards were processed according to the following one-step liquid–liquid extraction procedure. A 3-mL aliquot of biological sample was transferred to a 10-mL screw-capped glass tube and added with 100 µL IS (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 GC–FID screening analysis and then for GC–MS for confirmation of the obtained results.

Instrumental analysis. GC–FID analysis was performed with an HP 5890 series II equipment provided 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). Helium (Air Liquid, Madrid, Spain) carrier gas was delivered at a column head pressure of 22 psi, split ratio was 1:24, injector temperature was 280°C, oven temperature began at 40°C for 3 min, increased at 10°C/min to 280°C, and detector temperature was 300°C. The detector gases were hydrogen and air (Air Liquid), delivered at a flow rates of 40 and 400 mL/min,
respectively. Under these conditions, gasoline components including n-octylbenzene (IS) eluted between 1 and 15 min. Although no doubt was maintained about gasoline as source of poisoning, GC-MS analysis was performed with an HP 5971 mass-selective detector (Hewlett-Packard) for specific peak confirmation. The GC and column were as described. The MS was operated in the total ion chromatogram (TIC) mode (m/z 35-650), electron impact (EI) ionization energy was 70 eV, and transfer line and ion-source temperatures were both maintained at 280°C. Under these conditions extract ion chromatograms were obtained for ions: 91.00 (toluene), 106.00 (ethylbenzene and xylenes isomers), 120.00 (trimethylbenzene isomers), and 190.00 (n-octylbenzene, IS).

**Calibration curve.** Quantitative analysis was undertaken by GD-FID using a four point blood calibration curve (1, 25, 50, and 100 mg/L) and using m,p-xylene as reference peak for all gasoline calculations. Additionally, in the 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. R² value in the linear range was > 0.997. The limits of detection and quantitation were 0.3 and 1.0 mg/L. Accuracy was 77.6-98.3%, and intraday (n = 6) and interday (n = 10) precisions had a CV ≤ 5.4% between 1 and 100 mg/L.

### Table 1. Review of Reported Fatal Cases of Gasoline Poisoning with Analytical Data

<table>
<thead>
<tr>
<th>Age (years)/ Gender</th>
<th>Matrix</th>
<th>Gasoline Concentration</th>
<th>Manner of Death/Scene of Death</th>
<th>Author, Year, and Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/male</td>
<td>Lung</td>
<td>1 ml of fluid (obtained by distillation resembling petrol/195 g lung</td>
<td>Accidental inhalation/found dead, trapped in an overturned saloon car with his head in a pool of petrol</td>
<td>Ainsworth et al., 1960 (8)</td>
</tr>
<tr>
<td>63/male</td>
<td>Blood</td>
<td>1.0 %*</td>
<td>Accidental inhalation/found dead in the engine room of a fishing boat</td>
<td>Nagata et al., 1968 (19)</td>
</tr>
<tr>
<td>25/male</td>
<td>Heart blood*</td>
<td>51.5 ppm²</td>
<td>Accidental inhalation/found dead in a gasoline station, near a tank truck</td>
<td>Carnevale et al., 1983 (20)</td>
</tr>
<tr>
<td>44/male</td>
<td>Heart blood</td>
<td>0.051-0.447 mg/L³</td>
<td>Accidental inhalation/found dead in a taxi cab that had overturned and fallen into a dry riverbed</td>
<td>Ikebuchi et al., 1986 (21)</td>
</tr>
<tr>
<td>34/male</td>
<td>Blood</td>
<td>4.259 mg/kg*</td>
<td>Abuse inhalation/found dead face down in bed, sniffing</td>
<td>Kimura et al., 1988 (22)</td>
</tr>
<tr>
<td>59/female</td>
<td>Heart blood</td>
<td>Left ventricle: 19.2 mg/L**, Right ventricle: 13.3 mg/L**</td>
<td>Accidental inhalation/found dead in a fire, two empty containers of gasoline were beside the body</td>
<td>Matsubara et al., 1988 (23)</td>
</tr>
<tr>
<td>4 victims</td>
<td>Heart blood</td>
<td>Left ventricle, range: 7.3-34.9 mg/L**, Right ventricle, range: 5.2-27.3 mg/L**</td>
<td>Accidental inhalation/the victims were found dead in a fire</td>
<td>Matsubara et al., 1990 (24)</td>
</tr>
<tr>
<td>44/male</td>
<td>Blood</td>
<td>247 mg/L²</td>
<td>Accidental inhalation/found unconscious in his gasoline filled car. Chemical burns, bacterial pneumonia, and died of multiple organ failure on day 9</td>
<td>Matsumoto et al., 1992 (10)</td>
</tr>
<tr>
<td>36/male</td>
<td>Heart blood*</td>
<td>28.4 mg/L**</td>
<td>Accidental inhalation/found dead after inhaling the combustion fumes of a broken motor inside a 3-m well containing water. %COHb = 9%</td>
<td>Martínez et al., 2005 (16)</td>
</tr>
<tr>
<td>26/male</td>
<td>Heart blood*</td>
<td>18.0 mg/L², Peripheral blood 19.3 mg/L**</td>
<td>Accidental inhalation/discovered unconscious in a gasoline station, repairing a small box with tubes</td>
<td>Martínez et al., 2005 (16)</td>
</tr>
<tr>
<td>15/male</td>
<td>Heart blood*</td>
<td>38.3 mg/L², Peripheral blood 22.1 mg/L²</td>
<td>Abuse inhalation/found in cardiac arrest at home, sniffing gasoline and glue. %COHb = 6%</td>
<td>Martínez et al., 2005 (16)</td>
</tr>
<tr>
<td>73/female</td>
<td>Peripheral blood*</td>
<td>122.4 mg/L²</td>
<td>Accidental acute gasoline ingestion/suffered from senile dementia, found dead at home, a liquid smelling strongly of petroleum distillates was spilled from her mouth</td>
<td>Martínez et al., 2005 (16)</td>
</tr>
<tr>
<td>38/female</td>
<td>Heart blood*</td>
<td>22.3 mg/L**</td>
<td>Suicidal inhalation of motorbike exhaust/found dead after inhaling the combustion fumes of a motorbike inside the closed garage of her house. %COHb = 73%</td>
<td>Martínez et al., present case</td>
</tr>
</tbody>
</table>

* Two unknown constituents were used as reference peaks for gasoline quantitation.

† This author performed gasoline measurements also in other tissues.

² 2-Methylpentane was used as reference peak for gasoline quantitation.

³ Range obtained using different constituents (pentane and hexane isomers) as reference peaks for gasoline quantitation.

†† The sum of several constituents was used as reference peaks for gasoline quantitation.

²² This author also used several constituents as reference peaks for gasoline quantitation. We have chosen the results obtained using toluene as reference peak for gasoline quantitation because it is the more proximate to our method.

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Results and Discussion

We report here a lethal case of CO and gasoline exposure through the inhalation of vapors from a motorbike. Gasoline is a product consisting of a complex mixture of hydrocarbons and requires appropriate testing as it can be involved in forensic situations. The proposed analytical method allows a comprehensive toxicological screening for solvents, and other petroleum distillates in which gasoline is included. The screening method is based in the pattern recognition methods widely applied in many areas of forensic science (16,17).

In this report, gasoline components were easily identified by comparing chromatography of the gasoline standard to that of the samples using the GD-FID screening method. Figure 1 shows representative GC-FID chromatograms of gasoline standard, blank blood, gasoline-spiked blood, and blood obtained from the forensic case.

Although no doubt was maintained regarding the identity of the source of poisoning after analyzing the samples on the GC-FID, we also re-analyzed the heart blood sample of this case on the GC-MS for specific peak confirmation. Figure 2 shows a zoom of the TIC and mass ions obtained from the heart blood sample GC-MS under the described chromatographic conditions. Selected ions for target compounds and internal standard were detected by constructing chromatograms of molecular ions/principal fragment ions as described in our previous report (16).

The percentage of carboxyhemoglobin determined in the blood was 73%. The heart blood and vitreous humor gasoline concentrations were 22.3 mg/L and 1.0 mg/L, respectively. Regarding alcohol and other volatiles, abuse and therapeutic drugs screens the results were negative for all of them.

There is a paucity in the literature about methods to measure gasoline in biological samples and information about blood concentrations in fatalities is very limited (Table I). The gasoline concentrations obtained in the present case are comparable with other three reports of fatal inhalation described previously by us, in which the same analytical method based in the utilization of \( m,p \)-xylene as reference peak for quantitation was applied. Xylene \((m,p)\) constituent was selected for quantitation of gasoline because it is an abundant 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. On the other hand, it was not possible to know the exact relationship between xylenes and the other volatile hydrocarbons once the gasoline has been partly burned, passed through the exhaust of the motor bike and been absorbed into the body.

![Figure 1](https://academic.oup.com/jat/article-abstract/30/9/697/740721/Suicidal-Inhalation-of-Motorbike-Exhaust-Adding)

**Figure 1.** Representative GC-FID chromatograms of the forensic case: gasoline standard (75 mg/L) (A); blank blood (B); gasoline spiked blood (25 mg/L) (C); and heart blood obtained from the case (D). 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.
In one of them exposure to CO was suspected but COHb was not so high (9%) and therefore it was thought to have only partly contributed to death (16). The low concentration obtained for gasoline in vitreous humor also agrees with our previous experience (16) indicating that lipophilic substances do not concentrate in this biological fluid. Another approach to investigate the circumstances relating to CO intoxication by analyzing volatile hydrocarbons in the blood of cadavers has been reported (18). The victim was found inside a gasoline-fuelled automobile filled with exhaust gas, and only benzene, toluene, ethylbenzene, xylene isomers, and C6-aromatics were found as hydrocarbons. According to Morinaga et al. (16), these results are a result of the exhaust gas produced by petroleum-fuelled machinery that consists only of the more volatile components. These statements are consistent with the present case and with our previous results.

The hazard to health due to the fumes of volatile hydrocarbons, particularly in confined spaces, is well known (8,10,16,19–24). Surprisingly though, there have been only a few reported cases of fatal toxicity from gasoline that included autopsy findings or toxicologic studies (7–9,12,21,25). On the other hand, when a high % COHb is detected, the main origin of death is established, and other toxics are subsequently not analyzed. As a result, reports of combustible findings in vehicle-related CO deaths, self-immolation, or in fire victims are especially sparse (23,24,26), and the fuel is only analyzed from the containers, debris, or clothing recovered from the scene (27).

Motor vehicle exhaust accounted for more than half of the accidental CO deaths and is the most common method of intentional CO poisoning. Lethal concentrations of COHb can be achieved within minutes in the confines of a closed garage even by a small gasoline-powered engine (28–30). The quantity of CO produced depends on a number of factors, including idling speed, air-fuel ratio, compression ratio, and the presence of a catalytic converter. Catalytic converters are designed to convert CO to CO2 eliminating most of the CO if they are properly adjusted. Prior to their introduction, an idling engine could produce 7% CO, while the same engine in a vehicle traveling at 60 mph, with the carburetor adjusted to efficient operation, produced less than 0.5% CO. However small motorcycles, such as the one involved in our case, are not obliged to have a catalytic converter.

Forensic pathologists occasionally see deaths caused by inhalation of exhaust fumes in which the CO levels are in the normal range, probable as a result of the displacement of oxygen by CO2, the toxic effects of CO2, and the actions of other compounds in the exhaust. In fact, automobile exhaust fumes constitute a complex milieu with CO and also different concentrations of hydrocarbons based on the combustion efficiency (1,16,31).

CO and gasoline can act synergistically because they both originate behavioral aberrations that diminish the victim’s ability to rescue himself. Besides, acute mortality from CO appears to be due to ventricular dysrhythmias caused by hypoxic stress and central nervous system (CNS) depression or seizures, whereas gasoline can lead to arrhythmias due the lowering of the myocardial threshold to the arrhythmogenic action of epinephrine (4,5) and also produce CNS depression and respiratory failure (25,32,33). Furthermore, the effects of gasoline toxicity can be exacerbated by hypoxia from CO (34).

Levels of COHb that exceed 50% are considered to be incompatible with life, although lower may be associated with fatalities if there is some pre-existing pathology. In our case, however, the pre-existing cardiovascular disease could have been of minor importance in view of the high concentrations of COHb and gasoline in blood. On the other hand, CO exposure may be a contributing factor in deaths in which % COHb is 10–50% and hydrocarbons such as gasoline could be the main contributor such as we previously reported (16).

Based upon the toxicological data along with the information provided by the medical examiner, the cause of death was determined to be carbon monoxide and gasoline poisoning and the manner of death suicidal inhalation.

**Conclusions**

Fatal CO poisoning offers little difficulty in diagnosis. There is a history of exposure, a characteristic coloration, and COHb
determinations. On the other hand, in certain deaths involving CO, there is also exposure to other toxics, such as the those present in exhaust fumes, which are usually underestimated. As there is a paucity of recent references of gasoline concentrations in human cases, this article provides with new data about toxic concentrations and is a useful adjunct to the postmortem toxicological interpretation of fatalities involving gasoline. Besides, when only a COHb examination is performed in a CO intoxication, the cause of death can be determined but the origin of the incident cannot. Our analysis can be utilized to discriminate between situations related to CO intoxication when for instance the body is not found near the source of CO. Additionally, the evidence as to whether the victim was alive when the incident occurred could be seen not only after the finding of CO but also of gasoline in blood samples. Finally, a complete investigation to determine other co-intoxicants is essential in order to study all contributing conditions in the effort to study the mechanisms of toxicity. This is the first time that a fatalit with the combination of these two toxics has been reported in the literature with analytical data.

References


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