

Testing for gasoline acute poisoning in Watchdog by headspace-GC/MS a case report

Jamileh Salar Amoli, Jalal Hassan*, Shamsi Sadat Mosavi, Tahereh Ali Esfahani
Department of Toxicology, Faculty of Veterinary Medicine, University of Tehran, Tehran, (IRAN)
Toxicology and Animal Poisoning Research Center, Faculty of Veterinary Medicine, University of Tehran,
Tehran, (IRAN)
E-mail: jalalhassan@ut.ac.ir; jalalhassan1355@yahoo.com

ABSTRACT

A 2-year-old Watchdog was found dead in his house, where no vessel containing gasoline was found near him. The postmortem samples (stomach content and blood) were analyzed by gas chromatography–mass spectrometry and found to contain petroleum compounds. Gasoline showed tissue-specific damage to lung, we concluded that the dog's death was caused by acute toxicities with gasoline. © 2015 Trade Science Inc. - INDIA

KEYWORDS

Gasoline;
Dog;
Headspace;
GC/MS

INTRODUCTION

Petroleum-derived liquids such as gasoline may cause occasionally death in domestic, wild animals and huma^[1-3]. Domestic animals such as Watchdog may ingest petroleum products by burglar. Inhalation may occur in poorly ventilated areas where these chemicals have been used or stored. Gasoline and other petroleum products are not among the top animal poisons, but they can cause illness if ingested or exposed to the skin the most common petroleum. Petroleum hydrocarbon toxicosis is a severe and disease-like reaction that occurs when a dog is exposed to refined petroleum oil products, or ingests products of this type. For many complex samples, headspace extraction is the fastest and cleanest method for analyzing volatile components in dirty matrixes^[4,5]. Here, we describe a fatal case of gasoline poisoning and confirmation of gasoline in blood and stomach content of dog.

CASE HISTORY

A 2-year-old dog was found dead in his police station without any history of drug or illness (Kerman, Iran). The deceased was taken to University of Tehran (Faculty of Veterinary Medicine) for animal forensic autopsy. The postmortem interval was estimated to be 2 days. The following postmortem samples were collected for toxicological analysis: stomach content, and blood and stored at 4 °C until analysis.

MATERIALS AND METHODS

Materials

Two grams of each sample was transferred into a 10 mL glass vial with a PTFE-Silicon septum (Supelco) containing 5 mL 50% sulfuric acid and 12 mm×1.5 mm magnetic stirring bar. The sample vial was placed in a recirculation cell and maintained at a 60 °C using a water bath for 5 min. The

Full Paper

needle of the gas tight syringe was inserted into the internal tube above the extraction vial, pierced the vial septum and 0.50 mL of sample directly injected into the heated injection port of the GC-MS for analysis. Helium with a purity of 99.999% was used as carrier gas. The volatile compounds were analyzed using a GC/MS (Model 7890, Agilent Technologies, Palo Alto, CA, USA) equipped with a non polar column (J&W Scientific DB-5; 30 m, ID 0.25mm, film thickness 0.25 μ m). The column temperature was kept at 40 °C for 10 min, increased at 6 °C/min to 240 °C and isothermally maintained for 20 min. The mass selective detector (Model MSD 59556, Agilent Technologies, Palo Alto, CA, USA) was used in electron impact ionization mode. A mass range between 30 and 550 m/z was scanned. The mass spectra obtained were compared to the NIST Mass Spectral Search Program for compound identification.

Haematoxylin and eosin (H&E) staining

An aliquot of lung were quickly removed, rinsed with cold phosphate buffered saline (PBS) and then immersed in 10% neutral-buffered formaldehyde for 24 h, embedded in paraffin, and sliced into 5 μ m thickness. The sliced sections were stained with H&E, and examined under light microscopy.

RESULTS AND DISCUSSION

Histopathological changes of lung tissue

Histopathological findings revealed severe pulmonary hyperemia and intra alveolar hemorrhage. Diffuse interstitial, intra alveolar and intrabronchial edema is noticeable too (Figure 1, 2). This edema can cause death. Some bronchiole lumens were filled with amorphous materials along with red blood cells (Figure 3). For more details about changes in clara cells and pneumocytes, toluidine blue staining is required.

Gasoline is a complex mixture of alkanes, cycloalkanes, alkenes and aromatic hydrocarbons boiling between 0-200°C. The bulk of a typical gasoline consists of hydrocarbons with between 4 and 12 carbon atoms per molecule (commonly referred to as C4-C12). Virtually no alkenes or alkynes are present in gasoline (TABLE 1). Numerous constituents of gasoline including benzene, isobutane, cyclobutane, isopentane, and 2,2- dimethylbutane have all been shown to produce cardiac sensitization to administered sympathetic amines.

After injection of sample to GC/MS several unknown peaks were observed. The impact mass spectra of these obtained peaks were searched for in our computer library. These unknown peaks

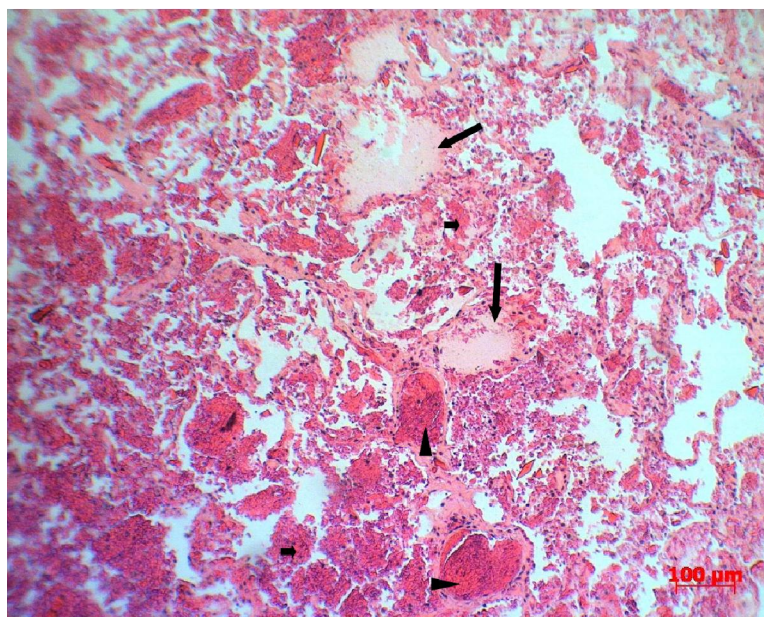


Figure 1 : Diffuse interstitial, intraalveolar and intrabronchiolar edema (long arrows), severe pulmonary hyperemia (arrow heads) and intra alveolar hemorrhage (short arrows) in lung tissue of the dog ($\times 100$ H&E)

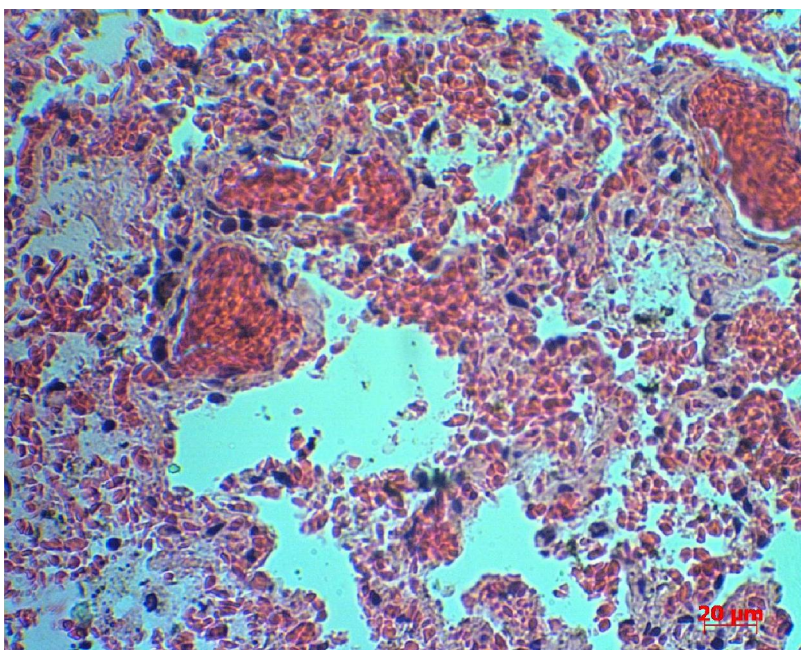


Figure 2 : Diffuse interstitial, intraalveolar and intrabronchiolar edema (long arrows), severe pulmonary hyperemia (arrow heads) and intra alveolar hemorrhage (short arrows) in lung tissue of the dog ($\times 400$ H&E)

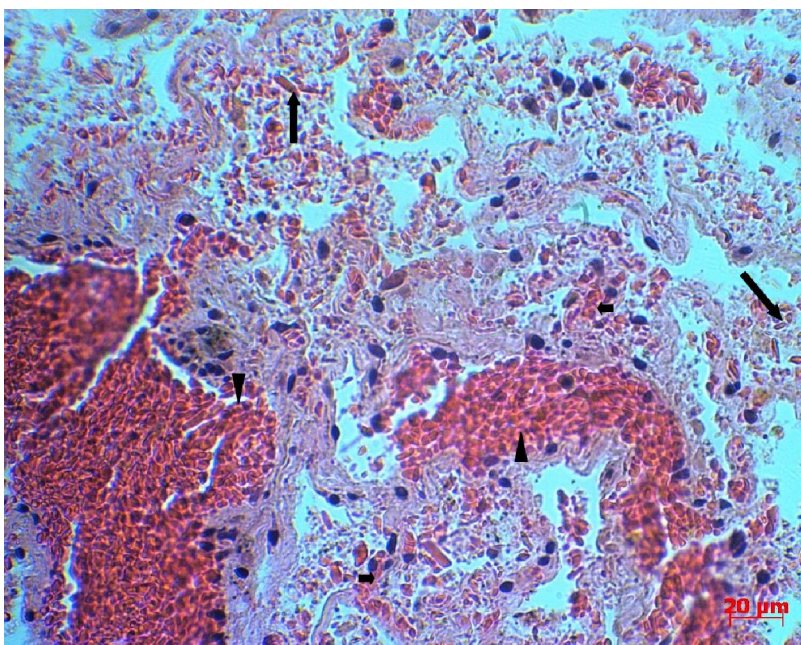


Figure 3 : Amorphous materials in bronchiols (long arrows), severe hyperemia (arrow heads) and intra alveolar hemorrhage (short arrows) in lung tissue of the dog ($\times 400$ H&E)

TABLE 1 : Typically compounds in gasoline

Typical Composition of Gasoline		
General Name	Examples	Percentage
Aliphatic - straight chain	Heptanes	30-50
Aliphatic - branched	Isooctane	20-30
Aliphatic - cyclic	Cyclopentane	20-30
Aromatic	ethyl benzene	20-30

TABLE 2 : Gasoline compounds concentration found in death dog case

Retention time (min)	Stomach content	Blood
1.54	-	Butane, 2-methyl
1.75	-	Methylthiomethane
2.31	Hexane	Hexane
2.70	Methylcyclopentane	
3.29	3-Methylheptane	3-Methylheptane
3.48	Hexane, 3-methyl	Hexane, 3-methyl
3.72	cis-1,3-Dimethylcyclopentane	cis-1,3-Dimethylcyclopentane
4.06	n-Heptane	n-Heptane
4.89	Methylcyclohexane	Methylcyclohexane
5.24	Cyclopentane, ethyl-	Cyclopentane, ethyl-
5.48	1,2,4-Trimethylcyclopentane	1,2,4-Trimethylcyclopentane
5.67	-	Dimethyl Disulfide
5.78	1,2,3-Trimethylcyclopentane	-
6.24	2,3-Dimethylhexane	-
6.51	2-Methylheptane	2-Methylheptane
6.66	-	-
7.23	1,3-Dimethylcyclohexane	1,3-Dimethylcyclohexane
7.67	1-Methyl-3-ethylcyclopentane	1-Methyl-3-ethylcyclopentane
8.17	Octane	Octane
8.50	Cyclohexane, 1,3-dimethyl	Cyclohexane, 1,3-dimethyl
9.29	cis-1-Methyl-2-ethylcyclopentane	-
9.57	2,6-Dimethylheptane	-
9.69	cis-1,2-Dimethylcyclohexane	-
9.88	Ethylcyclohexane	Ethylcyclohexane
10.07	1,1,3-Trimethylcyclohexane	-
9.88	Cyclohexane, 1,2,4-trimethyl-	-
11.20	-	Ethyl benzene
11.28	4-Methyloctane	-
11.38	2,6-Dimethyldecane	2,6-Dimethyldecane
11.69	Octane, 3-methyl-	-
12.38	Cyclopentane, 1-methyl-2-propyl-	-
12.72	o-Xylene	o-Xylene
13.09	Nonane	
13.47	trans-1-Ethyl-4-Methylcyclohexane	-
14.76	2,6-Dimethyloctane	-
Total aliphatic (straight chain+branched)	45	49
Total aliphatic (cyclic)	50	59
Total aromatic	5	8

were identified as hydrocarbon compounds (TABLE 2). Due to the large number of compounds present in automotive gasoline and the variation of gasoline composition from refinery to refinery, no attempt was made to quantitate the samples, but

according to normalized method semi quantitative of analytes were determined and compared with TABLE 1. Gasoline compounds were detected in the stomach content, and blood specimen (TABLE 2).

CONCLUSION

The cause of death in this case was acute poisoning from intake of gasoline to dog. However, the literature on experimental or actual dog exposure to gasoline vapors is extremely spare. Death resulting from gasoline inhalation is generally attributed to progressive medullary paralysis leading to respiratory failure.

Conflict of interest

I (we) certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

REFERENCES

- [1] Poklis; *J.Forens.Sci.SOC.*, Death Resulting from Gasoline “Sniffing”: A Case Report, **43**, 16 (1976).
- [2] E.B.Dede, H.D.Kaglo; *J.Appl.Sci.Enviro.Mgt.*, Aqua-toxicological Effects of Water Soluble Fractions (WSF) Of Diesel Fuel On *O. Niloticus* Fingerlings, **5(1)**, 93-96 (2001).
- [3] Jalal Hassan, Mohadeseh Izadib, Soma Homayonnejad; *Journal of brazilian chemical society*, Application of low density homogeneous liquid–liquid extraction combined with gas chromatography for the determination of TPHs and PAHs in semi-micro solid samples, **24(4)**, (2013).
- [4] Luigi Perbellini, Francesco Pasini, Serena Romani, Andrea Princivale, Francesco Brugnone; *Journal of Chromatography B*, Analysis of benzene, toluene, ethylbenzene and *m*-xylene in biological samples from the general population, **778**, 199–210 (2002).
- [5] J.A.Cruwys, R.M.Dinsdale, F.R.Hawkes, D.L.Hawkes; *Journal of Chromatography A*, Development of a static headspace gas chromatographic procedure for the routine analysis of volatile fatty acids in wastewaters, **945**, 195–209 (2002).