FATAL POISONING OF MONOCROTOPHOS PESTICIDE BY SUBCUTANEOUS ABSORPTION: A CASE STUDY

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ABSTRACT: Fatalities due to consumption of pesticides cases are common in developing countries. Monocrotophos is a type of highly hazardous organophosphate pesticide. We report a case of a woman who was found dead a couple of hours after pesticide poisoning due to absorbance through skin from India. Monocrotophos, an organophosphate pesticide, is basically a systemic and contact poison. It is highly hazardous and used in large quantities in India. In present study, monocrotophos was extracted using liquid-liquid extraction procedure with acetone as solvent. Thin-layer chromatography and mass spectrometric (GC-MS) techniques were used for the identification of monocrotophos in blood and skin patches. Monocrotophos was found in post-mortem blood and skin sample of deceased. None of pesticide was found in autopsied stomach, intestine, liver, kidney and spleen tissue. No other xenobiotics were detected in postmortem samples. The results showed that the cause of death was monocrotophos intoxication and the manner was accidental.

Keywords: Forensic toxicology, monocrotophos, dermal exposure, TLC, GC-MS.

Introduction

Globally, three million pesticide poisoning episodes occur annually. Fatalities due to consumption of pesticides cases (intentionally or unintentionally) are common in developing countries. In India, these cases would be estimated to be 600000 and out of which, 6000 fatal outcomes occur yearly (WHO). Pesticides are most common lethal poison in households due to the ignorance and carelessness about their shelf storage; and easy availability [1]. Organophosphate pesticides are used extensively worldwide and associated poisoning is a serious public health problems. Monocrotophos (Dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate; $C_7H_{14}NO_5P$; Figure 1) is a type of organophosphate pesticide, highly hazardous and used in large quantities in India.

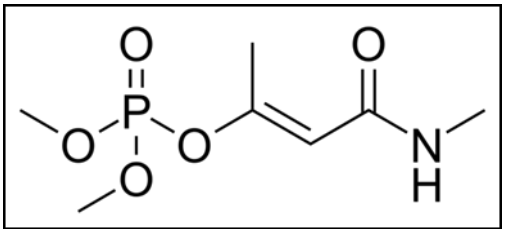

Figure 1: Structure of monocrotophos.

Monocrotophos poisoning cases have been reported from all part of India and have higher case fatality rates than other pesticides [2]. For example, on 16th July 2013, children in Gandaman village in Bihar (India) fell violently sick after a school lunch. Twenty-three children died within a few hours of eating in relation to monocrotophos poisoning [3]. Monocrotophos is highly volatile organic poison and can be purchased at many diverse types of retail outlets by the name as Azodrin, Apodrin, Bilobron, Monocron etc [4]. Experimental studies and occupational exposure specify that it is a systemic and contact poison. On contact with such poisons, it may cause skin to itch, blister or change colour and can pass through skin and get into the body [5]. Symptom of monocrotophos poisoning include convulsion, vomiting, abdominal pain, breathing problem and death (Acute poisoning).

Monocrotophos is known to be neurotoxin that affects the work of neurons in the body. Toxic effects of this compound produced due to action on central nervous system (CNS) of human body by blocking the activity of acetylcholinesterase enzyme and results in the preventing the breakdown of chemical acetylcholine [6]. WHO placed this highly acutely toxic compound in Class 1B – a category reserved for highly hazardous pesticide. It is a persistent organic pollutant and banned in many other countries including USA; however it is still used in India. Specifically, a large quantity of this compound used in cotton growing area. The easy availability in the Indian market makes it a major agent of self poisoning with high case of fatality rates [2].

Indeed, there are only a few cases of severe organophosphate toxicity due to skin absorption reported in medical literature. Peiris *et al.* reported

a case of a man who presented serious poisoning after monocrotophos skin absorption [7]. Dermal absorption of organophosphate pesticide should be considered a route through which severe exposure could occur. Transfer of pesticide chemical residue through the skin dependent on the physical attributes of the residue formed [8]. The LD_{50} value of monocrotophos for dermal exposure is 126 mg/kg for male rates, 112 mg/kg for female rats and 354 mg/kg for rabbits [9]. Literature survey showed a number of confirmed cases, where monocrotophos was determined to be present in body fluids and tissues like lungs and brain after ingesting a plant protective contain monocrotophos [10]. In a case study, a 19-year old male was splashed about 570 mL of monocrotophos on his bare chest and arms. He instantly attempted to wash out the material with water but did not remove his clothes. After 38 hr of exposure, the man was hospitalized due to toxicity symptoms. He was pale, sweaty profusely, confused, and unable to give any detailed history. Treatment with atropine and pralidoxime led to distinct recovery in 48 hr, and the patient was effectively symptom-free on the third day [11].

In fatal poisoning case, toxic compounds are primarily isolated from the biological materials such as viscera, blood, and tissue samples preserved after post-mortem [12]. It is a challenging task for a forensic toxicologist to isolate and determine the nature of suspected poison, as amount of poison available for estimation is just depiction of what leftovers of the original amount in body after death [13-14]. In present study, isolation and determination of monocrotophos in a case of fatal organophosphate poisoning resulting from subcutaneous absorption is discussed.

Case History

This is the case of a 47 years old woman. One day, when she was sleeping during night, a bottle of pesticide containing monocrotophos was fell down upon her back of right shoulder. The chemical absorbed through the skin to the body. She complained vomiting with breathlessness on next day. She was taken to hospital where she died on the same day during the medical treatment.

According to post-mortem report, a chemical burn injury present on the back of right shoulder extending to arm with redden base and black margin as a result of suspected poisoning by chemical substance. Autopsy blood, skin pieces, intestine, stomach, liver, spleen, and kidney tissues were submitted to Forensic Science Laboratory, Delhi for toxicological examination. Samples were extracted according to the procedures outlined below and analysed by Thin Layer Chromatography (TLC). TLC result was confirmed using GC/MS technique.

Materials and Method

Solvents and standard

All organic solvents used in study were suitable for pesticide residue analysis. An authentic neat standard (>95% purity) of monocrotophos was obtained from the Sigma-Aldrich (Catlog No. 46159). Acetone, and anhydrous sodium sulphate were purchased from Merck (Merck Life Science Pvt. Ltd., Mumbai, India). Hexane, chloroform, chloranil, sodium tungastate, anhydrous sodium carbonate and sulphuric acid were purchased from Merck (Merck Specialities Pvt. Ltd., Mumbai, India). All chemicals used in experiment were of HPLC grade.

Preparation of spraying reagent

For spraying reagant, 0.5 g of chloranil was dissolved in 100 mL acetone, while 10 g of anhydrous sodium carbonate was dissolved in 50 mL water.

Sample preparation

Samples were processed according to the liquid-liquid extraction procedure. Briefly, the portion of post-mortem blood sample (volume 10 mL) was deproteinised by using 5 mL of sodium tungastate solution, added with a few drops of sulphuric acid and shaken for 5 minutes, then filtered. The filtrate was transferred into a separating funnel and extracted with acetone. The extracts were dried over anhydrous sodium sulphate.

Skin patches of chemically affected area were taken into a conical flask. Skin tissue cut into fine pieces and minced carefully using distilled water. About 15 mL acetone added and mixture was shaking at intervals, kept for overnight and filtered. The extracts were dried over anhydrous sodium sulphate and the final volume adjusted to 10 mL.

Stomach and intestine tissues were homogenised with acetone using a high speed blender, left the tissue for the night as such and next day decant the solvent from the tissues. Decant solvent passed from anhydrous sodium sulphate to remove water content.

Tissues of liver, spleen and kidney were mixed with acetic acid and ammonium sulphate. The mixture was shaken at intervals, kept overnight in water bath (70°C) for digestion and filtered after cooling on the following day. The extract was taken into a separating funnel and extracted with acetone. The layer was then passed through anhydrous sodium sulphate to remove water content.

TLC analysis

TLC was performed on a 20 cm × 10 cm silica gel $60F_{254}$ TLC plate of thickness 200µm (Merck, Darmstadt, Germany). Samples were applied as spots approximately 2 cm apart and 1 cm from plate edge through 1 µL glass capillaries (Camlab, Cambridge, UK). Development was performed at room temperature in a horizontal chamber (Camag, Muttengz, Switzerland). The mobile phase used for separation were Hexane:Acetone (70:30, v/v) and left to allow the plate to dry. After spray (Chloranil reagent followed by sodium carbonate solution), spots were analysed and, compared the Rf value and colour of the spot with reference standard.

GC-MS analysis

GC-MS instrumental analysis was performed using a bench-top GC-MS system consisted of an Agilent 6890N gas chromatograph interfaced to an Agilent 5975 inert XL mass selective detector. The GC was equipped with a split/splitless injection port, operated in splitless mode (purge time set at 1 min.) maintained at a temperature of 250 °C. An Agilent 7683 auto sampler was used to inject 1.0 µL of each extract into the GC-MS. All chromatrophic separation were achieved using a 30 m DB-5MS fused-silica capillary column (5% Diphenyl- 95% methylpolysiloxane) having an inner diameter of 0.25 mm and a stationary phase film thickness of 0.25 microns. The oven temperature profile consisted of an initial temperature of 60 ºC ramped at 15 ºC/min to a final temperature of 250 °C which was held for 5 min. Mass spectrometry (MS) was operated in the electron impact (EI) ionisation mode with the electron energy set to 70 eV. MS was tuned and calibrated before sample run. All samples were initially screened using a cyclic scan mode where m/z value from 50 to 500 amu monitored in a period of 0.8 sec. GC/MS method as per DFS (Directorate of Forensic Sciences) manual used for the analysis of pesticides in samples [6] but a few of the parameters were modified for better results. The readings were compared with the NIST library Version 11.2 as well as Rt and mass spectra of the exhibits are tallied with the standard. Each sample was analysed twice.

Results and Discussion

Pesticide residues analysis is a complex process in biological samples and requires both skilled personnel and sophisticated equipment. TLC is simply carried out in any chemical laboratory, and it is a good technique for detecting known pesticide residue or for confirmation for tentatively identified compounds [15]. After spraying the reagents, pink colour spots were developed in TLC plate on the area where monochrotophos was separated (Figure 2). Portions of stomach, intestine, liver, spleen kidney, skin patches and blood were previously analysed by TLC and R_f values were tallied with monocrotophos standard shown in Table 1. A good method should determine the pesticide residue in biological samples even at low levels.

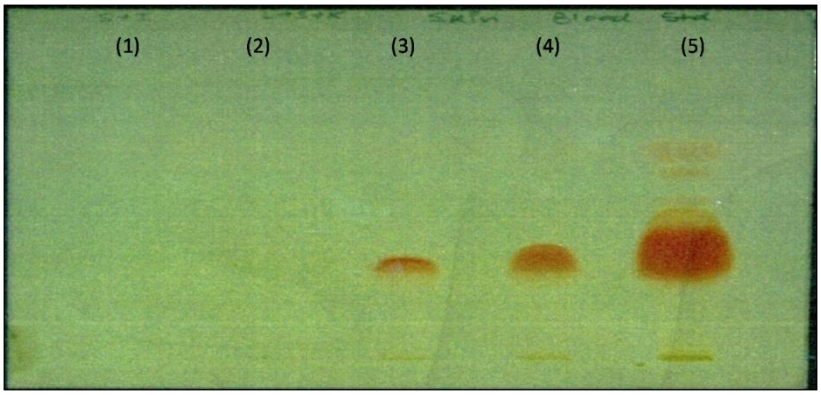

Figure 2: Thin layer chromatogram (1. Stomach and Intestine, 2. Liver, Spleen and Kidney, 3. Skin tissue, 4. Blood and 5. Monocrotophos Standard).

Table 1: Results of TLC.

Exhibits	Suspected R_f	Standard R_f	Colour of spot
Stomach + Intestine	Nil	0.3	Nil
Liver + Spleen + Kidney	Nil	0.3	Nil
Blood	0.3	0.3	Pink
Skin patches	0.3	0.3	Pink

Gas chromatography coupled with mass spectroscopy is a robust technique that has been proven and widely validated in toxicological screening [16]. In present study, exhibits of the case and standard have been examined and on the basis of TLC and GC-MS technique; blood and skin portion of the deceased were found to contain the monocrotophos. In other visceral part like portion

of liver, spleen, kidney, stomach and intestine, monocrotophos could not be detected.

All exhibits and standard of monocrotophos were analysed by GC-MS after TLC. The optimised conditions were used to validate the method for the qualitative analysis of monocrotophos. For the qualitative analysis of monocrotophos, the selectivity of the method and the characteristic ion fragmentation were investigated in present study.

Table 2 shows the results obtained from GC-MS. GC-MS analysis of blood and skin sample showed TIC peaks at RT 8.24 (Figures 3 and 4). Its mass spectrum m/z at 67.0, 97.1, 127.0, 223.1 which was closed to that of monocrotophos (Figures 5, 6 and 7). There were no peaks observed in other exhibits of stomach-intestine and liver-spleen-kidney. No endogenous interference was observed during the determination of monochrotophos. In present study, peak broadening in GC-MS experiment suggest that presence of biological matrix with an analyte could interfere in the mass spectrometric response [17].

Table 2: Results of GC-MS.

Exhibits	Rt (suspect)	Rt (standard)
Stomach + Intestine	Nil	8.24
Liver + Spleen + Kidney	Nil	8.24
Blood	8.24	8.24
Skin patches	8.24	8.24

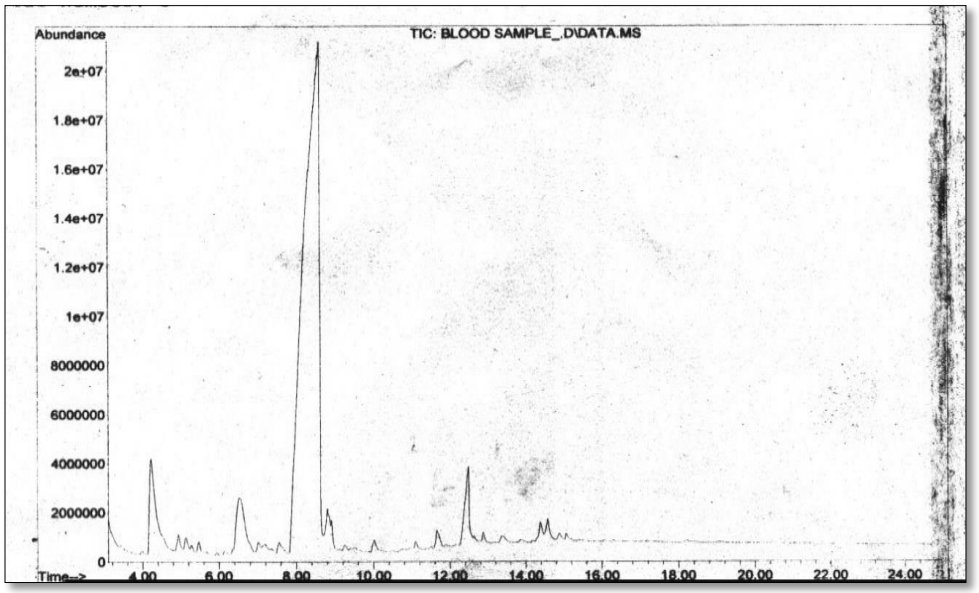

Figure 3: GC-MS Chromatogram of blood sample.

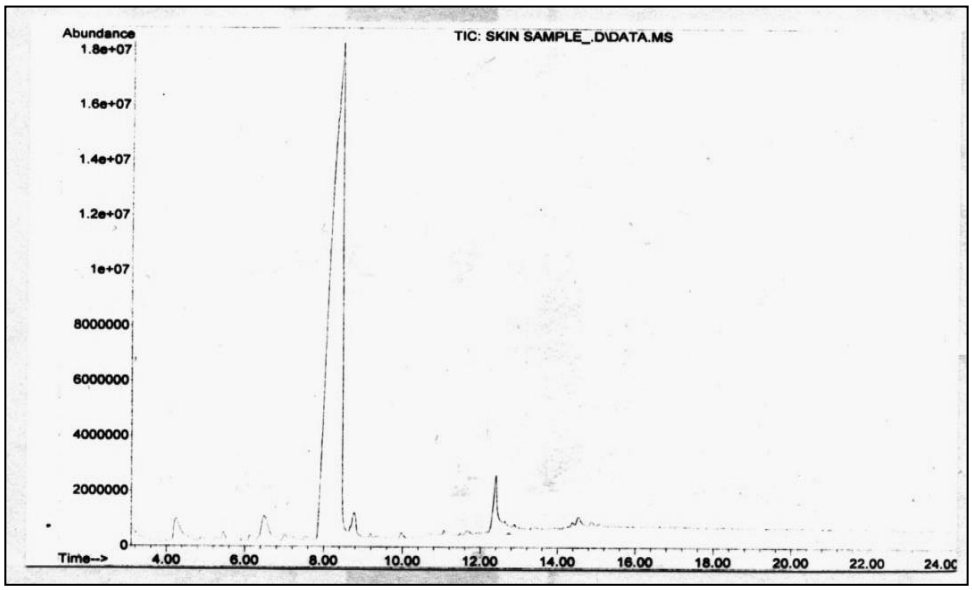

Figure 4: GC/MS Chromatogram of skin sample.

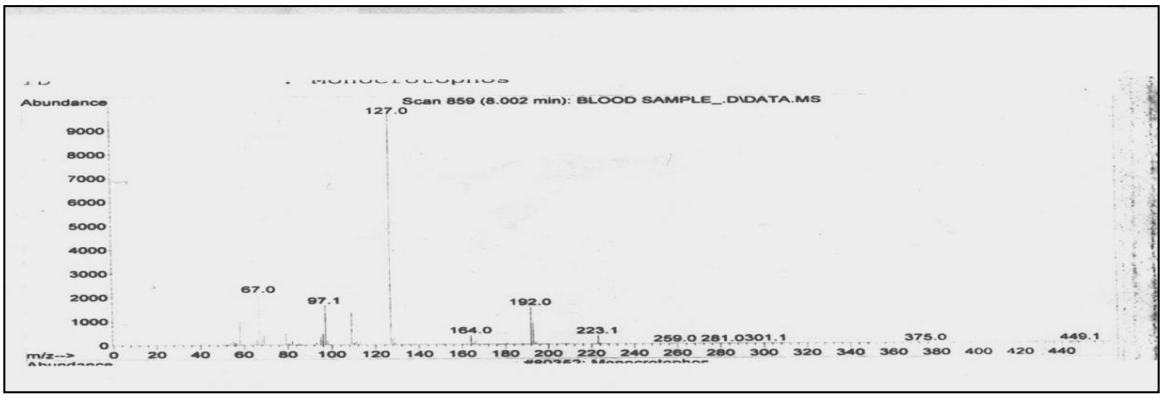

Figure 5: Mass spectra of monocrotphos peak at retention time of 8.24 min from the analysis of blood sample.

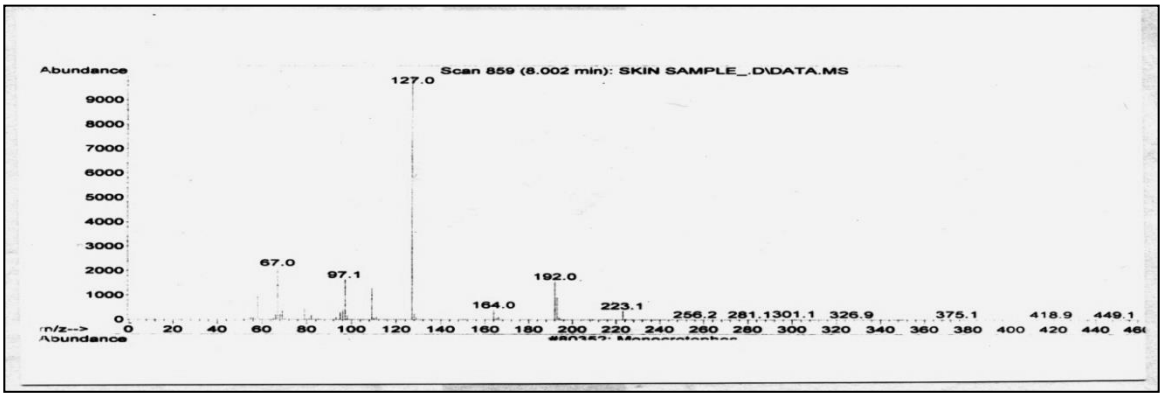

Figure 6: Mass spectra of monocrotphos peak at retention time of 8.24 min from the analysis of skin sample.

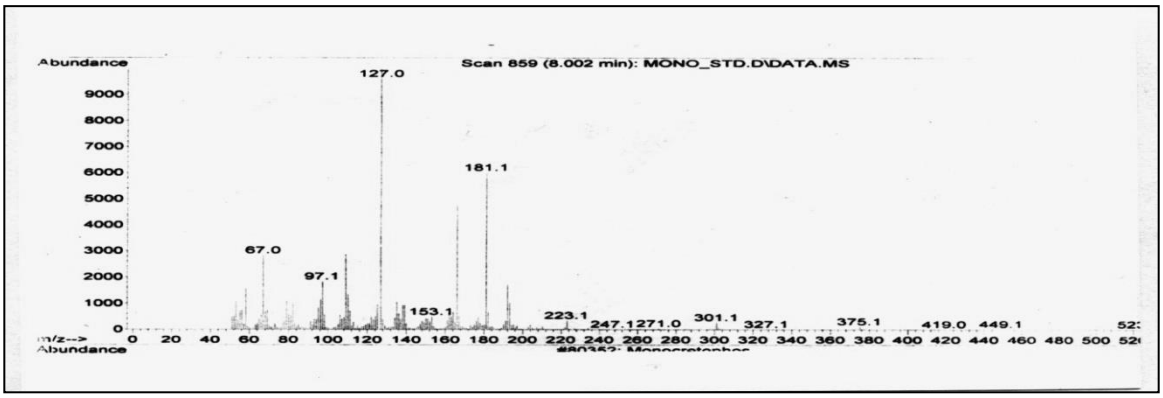

Figure 7: Mass spectra of monocrotphos peak at retention time of 8.24 min from the analysis of monocrotphos standard.

Conclusion

Finally, it may be concluded that monocrotophos, a systemic and contact poison, absorbs from skin to the blood, which block the neurotransmitence to the brain and cause fatal to that lady. It leads to death in short span of time. The results showed that the methods are suitable for the determination of monocrotophos in forensic toxicological analysis and clinical diagnosis. This study will be useful to prove the presence of a very toxic organophosphorous pesticide 'monocrotophos' in biological exhibits for investigating agencies and judiciary system.

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