Attempted Suicide by Ingestion of Chlorpyrifos: Identification in Serum and Gastric Content by GC-FID/GC–MS

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Abstract

A mild case of self-poisoning with a chlorpyrifos formulation following oral ingestion is reported. A 15-year-old female went to the emergency room after the ingestion of a product from a bottle marked with a label “Poison”. On admission, she was obtunded, with normal vital signs and a strong smell of solvent. Therapeutic measures included the application of decontamination procedures, oxygen, and gastric protectors. She had a good outcome with mild CNS depression and bradycardia. Two hours after ingestion, biological samples were collected in the emergency room and sent for analysis to our laboratory with instructions to investigate the presence of solvents. The serum and gastric content contained 5.3 and 9.4 pg/mL of unmetabolized chlorpyrifos, 4.6 and 6.9 pg/mL of toluene, and 2.5 and 7.9 pg/mL of butyl acetate, respectively. Small traces of other solvents and tetradifon were also detected. Toxicological analyses were negative for ethanol, other volatile solvents, and common drugs of abuse. The simultaneous determination of chlorpyrifos, toluene, and butyl acetate was performed using the combination of gas chromatography (GC)–flame ionization detection for screening analysis and GC–mass spectrometry for confirmation of the obtained results. The method provides an excellent and rapid tool for use in cases of pesticide poisonings, allowing the simultaneous detection of the pesticide and distillates in the performance of systematic toxicological analysis in forensic and clinical laboratories.

Introduction

Chlorpyrifos, O,O-diethyl-O-[3,5,6-trichloro-2-pyridyl] phosphorothioate (Figure 1), is an organic phosphorus pesticide introduced by Dow Chemical Company in 1965 as a broad-spectrum insecticide especially useful in controlling mosquitoes, flies, many foliage crop pests, household pests, and aquatic larvae (1). Chlorpyrifos is the active ingredient in many commercial insecticide formulations available in Spain (2).

Organophosphate insecticides inhibit both cholinesterase (ChE) and pseudo-cholinesterase activities. The inhibition of acetyl cholinesterase (AchE) causes accumulation of acetylcholine at synapse, and, as a result, an overstimulation of neurotransmission occurs. The mortality rate of suicide poisoning with these compounds is usually high, therefore early diagnosis and appropriate treatment is often life saving (3–5).

Chlorpyrifos undergoes oxidative desulfuration to form an oxon, which is 106 times more active as a cholinesterase inhibitor, and both chlorpyrifos and the chlorpyrifos-oxon are rapidly hydrolyzed to 3,5,6-trichloro-2-pyridinol (6).

Environmental or occupational exposure to chlorpyrifos is often estimated by quantitating the 3,5,6-trichloro-2-pyridinol metabolite in urine (7–10). However, the toxicity associated with chlorpyrifos is due to the oxon that inhibits AchE, whereas neither chlorpyrifos itself nor 3,5,6-trichloro-2-pyridinol inhibits AchE activity or that of other serine-dependent esterases or proteases (11,12).

Previous studies have reported that chlorpyrifos-oxon, when present, hydrolyzes to 3,5,6-trichloro-2-pyridinol in blood rapidly ($t_{1/2}$ rat blood $\times 10$ s, $t_{1/2}$ human blood $\times 55$ s). In order to halt the hydrolysis of the oxon to 3,5,6-trichloro-2-pyridinol, the blood samples can be treated with an acidic saturated salt

![Figure 1. Structure of chlorpyrifos.](https://academic.oup.com/jat/article-abstract/28/7/609/744566)
solution (containing ~2.5N acetic acid and a saturated amount of NaCl). This procedure, however, is not practical for clinical and forensic specimens. Furthermore, the oxon partially degrades to 3,5,6-trichloro-2-pyrindinol in the heated injection port of the gas chromatograph (GC) (13).

Other compounds can be present in organophosphate formulations and are responsible for part of the toxicity of these commercialized products. In fact, aromatic hydrocarbons, such as toluene and/or xylenes, and other additives could increase the risk of toxicity after ingestion of pesticide formulations (14).

Numerous cases of acute nonfatal (15-18) and fatal (19-21) poisoning because of the inhalation or ingestion of chlorpyrifos have been reported in the literature. However, there is a lack of chlorpyrifos poisoning cases published in which analytical findings are included (18-20). We report an unusual and unexpected case of acute self-poisoning with a chlorpyrifos formulation in a female teenager. The simultaneous determination of chlorpyrifos, toluene, and butyl acetate were performed using the combination of GC with flame-ionization detection (GC-FID) for screening analysis, and GC-mass spectrometry (MS) for confirmation of the obtained results, following a toxicological screening procedure used routinely in our laboratory. The analytical method was validated and described in this paper.

Case History

A consult was made to our Poison Control Center (PCC) from the Emergency Service of Hospital Rafael Méndez (Lorca, Murcia, Spain) concerning a 15-year-old female who had ingested, according to her words, a toxic product. At admission in the hospital, the girl appeared obtunded, with midriasis. The patient manifested a normal respiratory rate and a Glasgow coma score of 12. The abdominal exploration was anodyne. She had a strong odor of aromatic solvents. The patient was brought in by her parents after she had a discussion with a friend and tried to commit suicide impulsively but asked for help immediately. A nasogastric tube was introduced, and a thick liquid with a solvent odor was extracted. Serum, urine (less than 1 mL), and gastric samples were taken and sent for analysis to the National Institute of Toxicology with instructions to investigate the presence of solvents. Saline fluids, diuretics (furosemide), and metoclopramide were administered. Arterial pressure ranged from 113/71 mmHg to 88/65 mmHg during the period she stayed in the emergency room. Her heart rate ranged between 68 and 72 beats/min, but atropine was not required. Significant blood parameters revealed: pH 7.38; bases excess -1.7; pCO2 38.9 mmHg; pO2 111.5 mmHg; bicarbonate 22.6 mmol/L; leukocytes 7100/mm3; hemoglobin 12.8 g/dL; hematocrit 38.1%; glucose 131 mg/dL; urea 35 mg/dL; creatinine 0.5 mg/dL; sodium 141 mEq/L; and potassium 3.5 mEq/L. Oxygen FiO2 50 x 4 L/min was applied with an oxygen saturation of 96.8%.

Further research led to knowledge that the girl had ingested a compound from a container marked with the word "Poison" without the manufacturer label. She diluted it in a 1.5-L water bottle and ingested a third of it. She lived in a rural agricultural area, and her parents were agriculture workers. After psychiatric evaluation, she was discharged. She had no psychiatric history and was self-critical of the suicidal gesture.

Experimental

Materials

Sodium sulfate, diethyl ether, and methanol of analytical grade were obtained from Scharlau (Barcelona, Spain). Pesticide standards (chlorpyrifos and tetradinon) were purchased from AccuStandard (New Haven, CT). Aromatic hydrocarbons (toluene and xylenes) and n-octylbenzene (internal standard, IS) were purchased from Fluka-Sigma Aldrich (Buchs, Switzerland). Butyl acetate was purchased from Merck, (Darmstadt, Germany), and cyclohexanone was purchased from Scharlau. Stock solutions (1 mg/mL) were prepared by dissolving the appropriate amount of each substance in methanol. These solutions were used to prepare serum calibration standards of 0.1, 1, 3, and 5 μg/mL by adding toluene, butyl acetate, xylenes, cyclohexanone, chlorpyrifos, and tetradinon to a pool of citrated human serum samples from unexposed individuals provided by Comunidad de Madrid Blood Bank (Madrid, Spain).

Instrumentation

GC–FID screening analyses of the extracts were performed on a model 5890 series II GC equipped with an FID and an 7673A autosampler and linked to a 3396A integrator (all from Hewlett-Packard, Avondale, PA). A 25-m x 0.20-mm i.d. fused silica capillary column coated with cross-linked methylsilicone (0.11-μm film thickness, Hewlett-Packard) was employed. The oven temperature was held at 40°C for 3 min and then increased at a rate of 10°C/min to 280°C. The total chromatographic time, including 2 min of equilibrium time, was 29 min. The injection port and detector temperatures were 280°C and 300°C, respectively. The split ratio was 1:24. The carrier gas was helium (Air Liquid, Madrid, Spain) delivered at a column head pressure of 22 psi. The detector gases were hydrogen and air (Air Liquid), delivered at flow rates of 40 and 400 mL/min, respectively. Insert liners silanized with dimethylchlorosiliane/toluene (5:100) and packed with Supelco silanized glass wool (Supelco Park, Bellefonte, PA) were used.

GC–MS analysis was performed on a model 5971 mass-selective detector linked to an MS Chemstation series II (all from Hewlett-Packard). The autosampler, GC, and column were as

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<th>Table I. Parameters for GC–MS Analysis Using SIM</th>
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<td><strong>Compound</strong></td>
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<td>Chlorpyrifos</td>
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* Numbers in parentheses indicate relative abundances.
mentioned. The MS was operated in the electron impact (EI) ionization mode using an electron energy of 70 eV. Transfer line and ion-source temperatures were both maintained at 280°C. GC–MS was used in the full scan mode (35–650 amu) under the same chromatographic conditions and in the selected ion monitoring (SIM) mode. SIM chromatographic conditions were as follows: the oven temperature was held at 180°C for 1 min and then increased at a rate of 15°C/min to 255°C. The split ratio was 1:65.8. The total chromatographic time, including 1 min of equilibration time, was 7 min.

**Procedure**

All biological samples including the patient's serum and gastric content and serum control samples plus a reagent blank were processed according to the following one-step liquid–liquid extraction procedure: a 3-mL aliquot of each sample was transferred to a 10-mL screw-capped glass tube; 100 µL IS solution (n-octylbenzene methanolic solution of 100 µg/mL), 1 mL of diethyl ether (cold at 4°C), and 15 mg anhydrous sodium sulfate were added; and the tube was vortex mixed for 3 min and centrifuged at 4000 rpm for 10 min (at 4°C). The organic layer was then collected and transferred to a vial, and 3 µL was injected first for GC–FID screening analysis and later a full scan GC–MS analysis for confirmation of analyte identity using identical chromatographic conditions. GC–FID analysis was used for screening and quantitation of toluene and butyl acetate. Chlorpyrifos quantitation was carried out by SIM mode at m/z 314 (used for quantitation), 258, and 197 and by comparing the abundance with that of n-octylbenzene, the internal standard (m/z 190) (Table I).

**Validation of the method**

The method described here was also validated by adding toluene, butyl acetate, xylenes, cyclohexanone, chlorpyrifos, and tetradifon to human serum (100 mL) to achieve 0.05, 0.1, 0.3, 1, 3, and 5 µg/mL. The serum was sonicated for 5 min at room temperature and then submitted to liquid–liquid extraction using the extraction procedure and quantitations described.

Calibration curves were prepared with standard solutions of the analytes in diethyl ether. The concentrations were 0.1, 0.5, 1, 5, 10, and 20 µg/mL, and the concentration of IS was fixed at 10 µg/mL. Analytes to IS area ratios were measured, respectively, and the calibration curves were generated by least-squares linear regression. The regression lines were used to calculate the absolute recoveries (n = 6) of the studied compounds from spiked serum at six concentration levels.

The intra-assay precision was assessed at six concentration levels by the extraction and analysis on the same day of six spiked serum samples for each level.

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 baseline noise defined from 6 control samples.

The linearity of the method was checked by preparing six replicates of the calibration curves at six different concentrations, ranging from 50 to 5000 ng/mL, by addition of known amounts of the studied compounds to human serum.

**Results and Discussion**

**Assay characteristics**

A comprehensive toxicological screening for solvents was performed in the serum and gastric content samples. This included ethanol and other volatiles (methanol, acetone, n-propanol, and isopropanol) analysis by headspace GC–FID. The results obtained ruled out the presence of these volatiles. The serum and gastric GC–MS full-scan screening analysis using the described method revealed (in order of elution including traces) the presence of toluene; butyl acetate; para-, meta-, and ortho-xylene; cyclohexanone; chlorpyrifos; and tetradifon. Figures 2 and 3 show the full scan and a zoom, respectively, of the GC–MS chromatogram obtained in the GC–MS analysis of the patient's gastric sample. Figures 4–6 show the GC–MS mass spectra of analyte peaks eluting at 2.89, 3.66, and 20.48 min and the resulting match of the computerized library searches, respectively.

The previous GC–FID screening analysis was used for quantitation of toluene and butyl acetate. GC–MS SIM mode was used for quantitation of chlorpyrifos because of a matrix interference observed in a blank serum GC–FID screening analysis that overlapped with the analyte. Toxicological findings are shown in Table II. These quantitations were performed using serum calibration curves in the range 0.1–5 µg/mL. Small traces of tetradifon and other solvents (xylenes and cyclohexanone) were also detected.
In order to complete the comprehensive toxicological screening, the urine sample (less than 1 mL) was tested for propoxyphene, cocaine, and benzylecgonine, methadone, opiates, cannabinoids, benzodiazepines, amphetamine (and related compounds), barbiturates, and tricyclic antidepressants on a Hitachi 902 Automatic Analyzer using Cedia® reagents. The results were negative for all these drugs.

A summary of the validation data of this analytical method is shown in Table III. The method is precise, sensitive, and reliable for the studied analytes. Good linearity and excellent recoveries were obtained. One drawback of this method is that, under this extraction procedure, 3,5,6-trichloro-2-pyridinol was not detected, although this compound showed good chromatographic behavior.

For toxicological screening in our method, n-octylbenzene was chosen as the IS because it is not present in commercial products, as far as we know. Its chemical structure makes it suitable for use as an IS of a wide variety of compounds. According to our toxicological analytical experience, it has a very...
good extraction behavior (the relative recovery obtained for this standard was 103%, with a relative standard deviation of 5%).

The proposed analytical method allows the simultaneous determination of a wide variety of pesticides and additives, including petroleum distillates (Table IV), and the LOD was at least 100 ng/mL (signal-to-noise ratio = 3).

The application of this validated analytical procedure to any of the biological samples available in a poisoning incident should provide a rapid means of simultaneous identification of the type of organophosphorus toxicants and petroleum distillates so that a preliminary identification of these toxics can be established in less than 1 h. Consequently, it can help to determine the proper course of treatment for patients suffering from this type of poisoning.

Poisonings characteristics

This is an unexpected case of intentional ingestion of a substance by a female teenager who was managed as a solvent intoxication with nasogastric aspiration and gastric protectors. A relevant feature was the uncertainty of the etiologic agent. Although the occupation of the family could have suggested a pesticide or another agrochemical, the clinical features were not helpful in the diagnosis.

Surprisingly, the analysis of blood and gastric contents revealed the presence of chlorpyrifos, toluene, and butyl acetate, which is an unusual finding in a teenager suicide attempt. Suicide attempts are well reported, mainly among female teenagers, after family or emotional conflicts, as in this case. However, the most common method is the ingestion of psychoactive drugs (22–25). On the other hand, patients with intentional organophosphate ingestion are mostly men older than 18 years, mainly in the age group 30–50 years, especially in agricultural areas (data from our Poison Control Center and others) (4,26). In our country, chlorpyrifos can be found as an agricultural pesticide in concentrations ranging from 1.5% to 75% (2) or as an industrial pesticide in lacquer at 15%. As a household product, it is present in formulations to control mosquitoes, flies, ants, and cockroaches at concentrations of less than 1%. This low concentration makes household products less risky than the agricultural formulations, which can be liquid, wettable powder, or granules.

One significant aspect of this case was the good outcome of the patient, who had only mild signs and symptoms. The presence of cholinergic signs of organophosphate intoxication was minimal. This could be due to the rapid transport to hospital and therapeutic intervention. In fact, toxic effects of pesticides are dependent upon the inherent toxicity of the compound, susceptibility of the individual, as well as the dose and adequate treatment. This is the reason for the decreased pesticide mortality over the last 20 years (27). Hence, a reason for the mild signs of our patient could be a quick gastric aspiration, which avoided a complete absorption of the pesticide. Phosphorothionate insecticides such as chlorpyrifos are activated to chlorpyrifos-oxon, which is further detoxified by paraoxonase-1 (PON1) (28). The specific hydrolytic activity of PON1 paraoxonase/arylesterase enzymes in liver and blood provides a natural barrier against the entry of organophosphate toxins into the central and peripheral nervous systems. PON1 is a polymorphic enzyme in human populations, and different individuals express widely different levels of the enzyme (29). In addition, at higher doses of chlorpyrifos, differ-

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<th>Table II. Toxicological Findings</th>
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<th>Table III. Summary of Validation Data of Toluene, Butyl Acetate, Xylenes, Cyclohexanone, Chlorpyrifos, and Tetradifon from Fortified Serum Samples</th>
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<td>Compound (in order of elution)</td>
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<td>Toluene</td>
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<td>Butyl Acetate</td>
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* RSD = relative standard deviation.
Comparison of our analytical data with others obtained in similar circumstances is difficult. Most of the articles on chlorpyrifos including analytical results have been focused on pharmacokinetic and environmental exposures studies in experimental animals (7,13). However, there are a few published cases of poisoned patients reporting chlorpyrifos levels in biological specimens (18-20). Our data are similar to those of a poisoned patient who had ingested a concentrated solution (18). Unfortunately, the contents of the bottle from which our patient drank were not sent for analysis.

Also of consideration was the contribution of toluene to our patient’s clinical features. Gastric and serum analysis identified toluene as the predominant petroleum distillate. Butyl acetate is used as a synthetic flavoring ingredient in the production of fruits flavorings, as well as in manufactured products (e.g., lacquers, nail polishes and removers, artificial leathers, perfumes, photographic films, industrial solvent mixtures, etc). It can be irritating and narcotic in high concentrations (31). In this sense, identification of these additives helps to explain the clinical features present in the patient besides the ones due to the active ingredient itself.

Conclusions

A suicidal attempt due to ingestion of chlorpyrifos in a teenager is reported. The pesticide and additives were simultaneously and quickly identified and quantitated in serum and gastric specimens by a GC-FID/GC-MS screening validated method in daily use in our laboratory. The proposed analytical method allows the simultaneous determination of a wide variety of pesticides and additives, including petroleum distillates. This is crucial to solve poisoning cases such as suicide attempts in which the poisoning source is uncertain. In addition, due to the paucity of chlorpyrifos analytical data, our report offers information that can help to interpret results in further forensic cases.

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