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Short communication

# Determination of chlorpyrifos in air, leaves and soil from a greenhouse by gas-chromatography with nitrogen–phosphorus detection, high-performance liquid chromatography and capillary electrophoresis

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**Abstract**

Chlorpyrifos was determined in air, leaves and soil in a greenhouse in order to establish performance differences between gas-chromatography with nitrogen–phosphorus detection (GC–NPD) and high-performance liquid chromatographic and capillary electrophoretic methods and to assess the farm workers' risk of overexposure due to air exposure and/or skin contact with this compound. Results obtained indicate that the three analytical techniques, with the specific procedures described, can be used, although only GC–NPD provides an operative limit of detection in air. Chlorpyrifos levels in air are dependent on time and greenhouse ventilation, whereas it remains for a long time on leaf surfaces and soil. As a consequence, specific instructions can be established for farm workers in order to avoid skin and respiratory exposure to chlorpyrifos. 

*Keywords:* Air analysis; Soil; Plant materials; Environmental analysis; Chlorpyrifos; Organophosphorus compounds; Pesticides

## 1. Introduction

Chlorpyrifos is an organophosphorous insecticide widely used for pest control in agriculture and, to a lesser degree, for indoor applications. A wide range of noxious effects of the organophosphates on humans have been described. The immediate effect of an acute exposure is the accumulation of acetylcholine at the receptors, giving rise to the characteristic symptomatology of the acute organophosphorous poisoning. The signs and symptoms found in people after acute exposure have been extensively described [1]. The chemistry of chlorpyrifos and its metabolism, cholinergic toxicity (also due to its active metabolite chlorpyrifos oxon) and low reproductive toxicity [NOEL (non-observed effect level) 5 mg $kg^{-1}$ $day^{-1}$ in the rat], have been fully reviewed [2].

Chlorpyrifos dermal $LD_{50}$ in rats is 202 mg/kg, [3] TWA-TLV (ACGIH) [4] has been set at 0.2 mg $m^{-3}$, with 'skin' notation, meaning that dermal absorption may pose a significant occupational health risk if manipulated under improper conditions, specially when used as an agricultural emulsifiable concentrate (EC) formulation, whose organic solvents may strongly enhance skin penetration.

Safety measures for farm workers (mainly small-

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holders) are very poor. Many of them do not strictly follow the manufacturer's directions for use of the EC formulations containing 48% (w/v) of chlorpyrifos marketed in Spain, which strongly recommend that it is not to be applied by cold fogging or atomising. It was therefore decided to study air, leaf and soil concentrations after an experimental application (not intended for pest control) was carried out in a greenhouse with an ultra-low-volume application system (ULV) in the coastal zone of El Maresme (autonomous region of Catalonia, Spain).

Chlorpyrifos analysis is carried out conventionally by gas chromatography (GC) or by high-performance liquid chromatography (HPLC) [5]. The analysis of greenhouse samples was carried out by GC and HPLC, and new analytical methods were developed for capillary electrophoresis (CE) by using the anionic additives, sodium dodecylsulfate (SDS) [6] or bile salts [7], or the cationic additive tetraoctylammonium bromide (TOAB).

The aims of this paper were: (1) to address the analytical aspects of chlorpyrifos determination in air, leaves and soil, highlighting performance differences between GC, HPLC and CE; and (2) to assess the farm workers' risk of overexposure due to airborne chlorpyrifos or/and skin contact.

## 2. Materials and methods

A 2.5-l volume of an emulsion was prepared by mixing 100 ml of a commercial 48% (w/v) chlorpyrifos EC formulation with 2.4 l of tap water. This was immediately poured into the tank of the ULV equipment, just before starting the experiment. This amount of formulation was about 1/3 of the recommended dose for the treatment of the surface (340 ml of the formulation). The ULV equipment (Nebula ULV, conic system) operates as an automatic system. The nebulized solution of pesticide coming out of the nozzle is spread by the nebulizer fan and an auxiliary fan.

The experiment was carried out in a rectangular greenhouse (104 m×22 m; 9300 $m^3$ volume) containing tomato plants. All the main facilities (air heating, irrigation, roof vents opening) were computer-controlled. Steps were taken in order to minimise workers' risk of overexposure during the experimental period (1 week).

### *2.1. Sample collection and treatment*

A total of 60 air samples, 60 leaves samples and 10 soil samples were taken.

#### *2.1.1. Air samples*

Samples collectors were placed at six locations, on top of plant rows, 1 m over the floor. Each sampler consisted of a 'cassette' with a fibreglass filter (5-mm D) and two sorbent tubes (SKC, containing 100/30 mg XAD-2 each) attached to a pump that operated a 1 $l\,min^{-1}$ for 60, 120 or 180 min according to the anticipated gradient of concentrations within the experimental period. The fibreglass samples were extracted with 5 ml of toluene–acetone (90:10, v/v) containing triphenyl phosphate as internal standard (I.S.) and the sorbent tubes with 2 ml of the same solution. Both were extracted for 1 h with occasional agitation and were analysed by GC [8]. For HPLC and CE analysis, samples were concentrated to dryness under nitrogen and redissolved in 250 μl of the mobile phase (acetonitrile–water, 80:20, v/v).

#### *2.1.2. Leaves*

The samples were taken from plants very close to the locations of air samples and at the same time. Each sample consisted of 10 discs from leaves of the external part of the plant, taken with a cutting punch (22-mm D). Samples were extracted with 3 ml of acetone for 1 h including in this time 20 min of ultrasonic bath. Afterwards the extracts were analysed by GC. For HPLC and CE analysis, 2 ml of sample were concentrated to dryness and redissolved in 1 ml of mobile phase (acetonitrile–water, 80:20, v/v).

#### *2.1.3. Soil*

Samples were collected close to location three. A 25-g amount of each surface soil sample was extracted with 25 ml of methanol with mechanical shaking for 1 h [9]. A 5-ml aliquot from each sample was concentrated to dryness under nitrogen and then redissolved in 200 μl of methanol. The recovery of chlorpyrifos from soil samples was 76%.

### 2.2. Analytical conditions

The GC study was carried out using a Hewlett-Packard 6890 gas chromatograph equipped with nitrogen–phosphorous detector (NPD) and a SPB-5 capillary column, 30 m×0.25 mm I.D. and 0.25 μm of film thickness. Temperatures were: column, initial 160°C (3 min) and final 250°C (2 min); rate 7°C $min^{-1}$; injector 280°C; detector 300°C. Helium at a linear speed of 19 $cm\ s^{-1}$ was used as the carrier gas, and hydrogen (3 $ml\ min^{-1}$) and air (60 $ml\ min^{-1}$) were used for NPD.

For HPLC analysis, a Spectra-Physics SP-8700 apparatus operating at 200 nm and a HPLC Waters 600S controller with a 996 photodiode array detector were used with a reversed-phase $C_{18}$ Kromasil 100, 5 μm (250×4.6 mm I.D.). Flow-rate was 1.5 $ml\ min^{-1}$. For chlorpyrifos detection at 200 nm, a water–acetonitrile (20:80, v/v) mobile phase was used. For photodiode array detection of chlorpyrifos and its metabolite, a water–acetonitrile (75:25, v/v) mixture containing 0.04% of phosphoric acid was used.

The CE system consisted of an integrated ISCO 3850 equipped with a fused-silica capillary column (540×0.05 mm I.D.) and an on-column UV detector positioned 40 cm from the anode. The sample was introduced into the system by vacuum injection (0.05 p.s.i.) for different injection times (20 to 3 s corresponding to 20 and 3 nl) (1 p.s.i.=6894.76 Pa). In the case of anionic additives, 50 m*M* solutions of the surfactant sodium dodecylsulfate (SDS) or sodium deoxycholate (NaDCh) were used in a 10 m*M* $Na_2HPO_4$, 6 m*M* $Na_2B_4O_7$, 25% acetonitrile buffer solution. In the case of cationic additives, a 10 m*M* tetraoctylammonium bromide (TOAB) in water–acetonitrile (50:50, v/v) solution was used.

### 2.3. Calibration

The linearity of the NPD system was studied and a calibration curve plotted for quantified chlorpyrifos. Standard solutions obtained by weighing and dissolving appropriate quantities of chlorpyrifos in toluene–acetone containing triphenyl phosphate as I.S. were used. The linearity range for quantified chlorpyrifos by GC–NPD, calculated from area/mass vs. injected mass graph was 2–60 ng. The calibration equation by GC–NPD was $y=1.17587x+1.0054\cdot10^{-1}$, $r^2=0.999$ where $y$ is the area and $x$ is chlorpyrifos concentration in $\mu g\ ml^{-1}$. Calibration curve for HPLC was $y=34400x+8680$, $r^2=0.999$ in a range of 2–25 $\mu g\ ml^{-1}$.

### 2.4. Chemicals

Chlorpyrifos was purchased from Dr. Ehrenstorfer (Augsburg, Germany). Reagents for the preparation of the mobile phases and buffer solutions were: analytical reagent grade $Na_2HPO_4$, SDS from Merck (Darmstadt, Germany), sodium tetraborate from Panreac (Barcelona, Spain), NaDCh from Sigma (St. Louis, MO, USA), TOAB from Aldrich (St. Louis, MO, USA), HPLC-grade acetonitrile from Romil (Leicester, UK), HPLC-grade methanol, toluene and acetone from Carlo Erba (Milan, Italy) and triphenyl phosphate (I.S.) from Sigma-Aldrich (Gilingham, Dorset, UK).

## 3. Results and discussion

### 3.1. Chlorpyrifos concentrations in air, leaves and soil samples

Chlorpyrifos concentrations time-course in air, leaves and soil samples show different patterns depending on the sample matrix.

The chlorpyrifos concentrations in air decrease quickly with time. Under above-mentioned conditions, the concentration has decreased by 50% only 3 h after the application, and in samples taken after the 2-h ventilation period of the greenhouse (11 h after insecticide application) chlorpyrifos was not detected.

The decrease of the chlorpyrifos concentration in the case of leaves was not so remarkable as in the air samples. A decrease of 50% from the initial concentration occurred in roughly 24 h. Nevertheless, chlorpyrifos was still detected up to 84 h after the application. The high variability of the results in leaves seems to be inherent to sample variation.

Two contrary tendencies were observed in the soil concentrations time-course. After the application, soil chlorpyrifos concentration increased from 25 to

60 mg $kg^{-1}$ in 3 h, a fact that might be attributed to chlorpyrifos deposition from the air. When the soil–air equilibrium is reached, soil chlorpyrifos starts to decrease slowly, probably due to the combination of vaporisation [10] and degradation effects [11]. Almost 60 h elapsed before the soil level decreased to the initial value. In spite of the strong chlorpyrifos adsorption in soils [12], the pesticide was not detected in soil samples taken 60 days after application.

From those results, it can be concluded that chlorpyrifos levels in the air are dramatically dependent on time and greenhouse ventilation. So, workers should not go into the greenhouse before complete greenhouse ventilation and aeration. However, chlorpyrifos remains for a long time on leaf surfaces and soil. As a consequence, special preventive measures should be taken in order to avoid excessive skin contact with leaves and other elements.

### *3.2. Comparison between GC, HPLC and CE*

The three analytical techniques (GC, HPLC and CE) were applied to the analysis of residual chlorpyrifos in air, leaves and soil greenhouse samples.

GC and HPLC are the main techniques used for chlorpyrifos analysis [6] and the analytical conditions are well documented. This is not the case for CE, a new technique where analytical methodologies need to be developed for the determination of chlorpyrifos in different types of samples. As chlorpyrifos is a neutral compound, buffer additives, such as tensoactives, are required for a proper separation. In the present work, two different anionic additives (SDS and bile salts) and one cationic additive (TOAB) were tested. From the results obtained different conclusions can drawn depending on the sample matrix.

Air samples could be analysed by the three instrumental techniques, but differences in the limits of detection (LOD) (0.01, 0.2 and 1 mg $m^{-3}$ for GC, HPLC and CE respectively) were observed. Due to those LOD values, chlorpyrifos could be detected by GC in any air samples up to 24 h after application, while it could only be detected up to 8 h and 3 h after application by the HPLC and CE analysis, respectively. Such limitations made GC (see Fig. 1) a more convenient technique for such low levels.

Unlike the GC chromatograms of leaf samples, which were very clean and showed only the peak corresponding to the analyte with a LOD of 0.025 mg $cm^{-2}$, HPLC chromatograms of leaf samples showed several compounds at retention times higher than that of chlorpyrifos, involving long analysis times. Such an observation and the higher LOD obtained for HPLC (0.5 mg $cm^{-2}$) made GC the most convenient technique for this type of analysis. CE analysis with SDS showed elution of different compounds interfering with chlorpyrifos, which migrate close to the micellar time. This problem was overcome by using NaDCh, but resolution was still poor.

HPLC analysis of soil extracts showed good results (see Fig. 2), the concentrations being higher

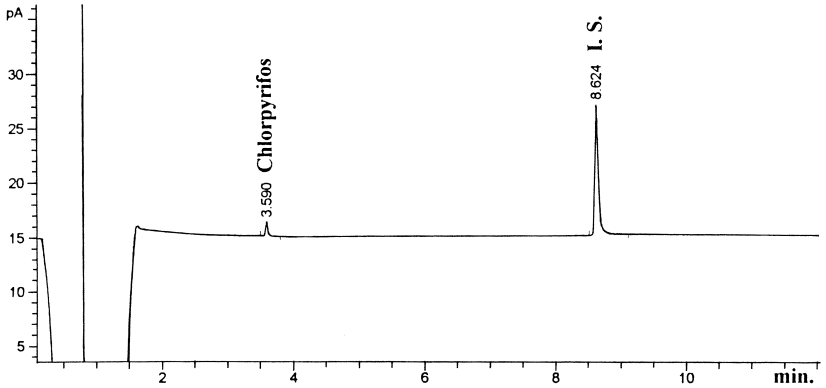


Fig. 1. GC chromatogram of an air sample

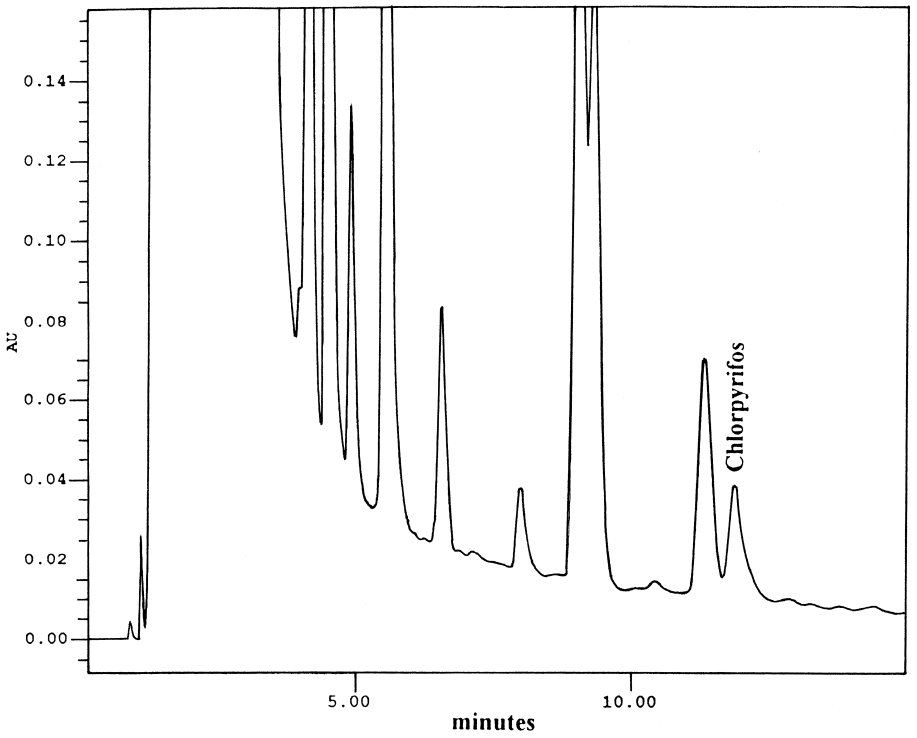


Fig. 2. HPLC chromatogram of a leaf sample.

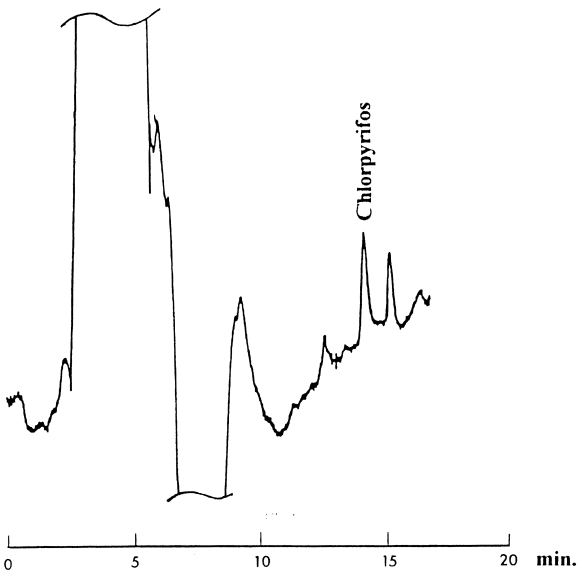


Fig. 3. CE electropherogram of a soil sample.

than the LOD (0.05 mg $kg^{-1}$) for all the samples collected up to 84 h after application. CE technique using anionic additives (SDS or NaDCh) was not convenient for soil analysis, since when calcium and magnesium cations present in the sample came into contact with the dodecylsulfate and cholate anions, the corresponding salt precipitated clogging the column and blocking the electroosmotic flow. In order to avoid on-column precipitation, a cationic modifier (TOAB) which was able to make solvophovic interactions with hydrophobic compounds, was used (see Fig. 3). However, the sample interference observed made sample dilution before injection necessary, increasing the LOD of the technique.

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