

# Analysis Of Pesticide Chemicals From Human Blood Samples

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**Abstract**

Pesticides are any compound or combination of substances that have been employed to prevent, eradicate, repel, or mitigate any pest. In the fields of forestry, agriculture, and public health, pesticides and synthetic pyrethroids have been utilized recently. Data on the blood level for pesticide analysis can be collected at a convenient time, mainly after working hours. The trained laboratory technicians took 4mL of blood samples with a sterilized syringe from the participants, transferred them into polyethylene tubes, and then kept them in an ice-cold box. Then it was transported to the laboratory of the department and stored at - 20°C until further study. After the Cleanup of pesticide residues, the samples were prepared in 1.5 mL of hexane and subjected to GC-ECD or GC-MS for pesticide analysis.

**1. Introduction**

According to (Afata et al., 2022, Marrs and Ballantyne, 2004), pesticides are any compound or combination of substances that have been employed to prevent, eradicate, repel, or mitigate any pest. In the fields of forestry, agriculture, and public health, pesticides and synthetic pyrethroids have been utilized recently (Afata et al., 2021)(Huong et al., 2020, Abdelkhalek et al., 2017, Coats, 1990). Many harmful, persistent, and outlawed pesticide chemicals are widely utilized in poor countries, which poses a major risk to human health and the environment(Ecobichon, 2001, Lushchak et al., 2018)

**2. Data collection and blood sampling**

Data on the blood level for pesticide analysis can be collected at a convenient time, mainly after working hours. The trained laboratory technicians took 4mL of blood samples with a sterilized syringe from the participants, transferred them into polyethylene tubes, and then kept them in an ice-cold box. Then it was transported to the laboratory of the department and stored at - 20°C until further study.

**3. Cleanup of pesticide residues**

4mL of blood samples were diluted with 25mL of distilled water and 2mL of saturated brine solution and then transferred to a 60mL separator funnel. Then extracted by adding 10mL acetone and 10mL hexane in the same ratio three times by shaking the mixture found in the separator funnel by hand for approximately three minutes with occasional releasing pressure. Finally, it was allowed to stand until it formed separate layers. After that, the upper (non-polar) layers were obtained by removing the lower (polar) layers. This has been repeated three times. The three combined extracts were passed through anhydrous sodium sulfate to eliminate a few residual polar solvents. The USEPA (USEPA, 1980) 3620B procedure was applied to clean up the extracts; florisil was activated overnight at 130°C and chilled in desiccators until used. One gram of florisil was predetermined through calibration and added to column chromatography. After adding anhydrous sodium sulfate to the top of the florisil column up to 0.5 cm, the column was cleaned with n-hexane and disposed of. Subsequently, the extracts were gradually added to the column tops. 10 mL of hexane was added first; 8.5 mL of hexane was added next; 1.5 mL of diethyl ether was added third; 5 mL of hexane was added fourth; and 5 mL of diethyl ether was poured gradually into column chromatography. Eventually, the eluent was dried out using a rotating vacuum evaporator and was contained in a single container. Finally, samples were prepared in 1.5 mL of hexane and subjected to GC-ECD pesticide analysis.

**4. Chemicals and reagents**

High-purity organic solvents can be employed, such as magnesium silicate or florisil with a mesh size of 60–100, saturated brine solution (99%), anhydrous sodium sulfate (99%), acetone (99%), diethyl ether (99%), and n-hexane (99%). The standards used for pesticide chemicals should be high-quality

**5. Chemical analysis**

The gas chromatography-electron capture detector has determined the pesticides by (GC-ECD, Agilent Technologies7890A) with an autosampler.

HP-5 capillary column of 30 m x 0.25 mm i.d. x 0.25 µm film thickness, was used in combination with the following oven temperature program: The initial temperature was 80°C, ramp at 30°C min<sup>-1</sup> to 180°C, ramp at 3°C min<sup>-1</sup> to 205°C, held for 4 min, ramp at 20°C min<sup>-1</sup> to 290°C, held for 8 min, ramp at 50°C min<sup>-1</sup> to 325°C. The total time of the GC run was 27.92 min. Helium (99.999% purity) was used as a carrier gas at a flow rate of 20 mL min<sup>-1</sup> and high-purity nitrogen (N<sub>2</sub> = 99.999%) as a makeup gas at a flow rate of 60 mL min<sup>-1</sup>. An aliquot of 1 µL was injected in split mode with a 50:1 split ratio and a 280°C injection temperature. With an electron capture detector (µ-ECD) working at a temperature of 300°C, the pesticide residues were identified.

## 6. Quality assurance and quality control

For every five samples, procedural blanks were examined for quality control purposes to look for contamination or interference from the solvent, glassware, and other tools used throughout the entire analytical process. Using the external standard addition approach, the chosen OCPs and SPs were quantified. Five distinct pesticide standard concentrations were spiked within the range of 10, 1, 0.1, 0.01, and 0.001 mg/L to obtain the calibration curves. For every pesticide under investigation, the regression coefficient of the standard curve was higher than 0.9994. To determine the limit of detection, the concentration of the analyte in the sample that resulted in a peak with a signal-to-noise ratio of three was employed. After measuring the samples with the lowest concentration level at which the peaks of the pesticides under study were observed, signal-to-noise ratios of 1:10 were used to compute limits of quantitation (LOQ) (Nassar et al., 2016).

## 7. Statistical analysis

The collected data were defined and entered SPSS software version 24.0 (IM Corp., Armonk, NY, US). First, a descriptive analysis of socio-demographics, working duration, training conditions, complete PPE use, following the labeled instructions, activities performed during spraying, condition of the equipment, and spraying against the wind variables were used to characterize the study population. To get a valid result, mean values, detection frequencies, and selected percentiles were used for descriptive purposes. Detection frequencies were calculated for the entire study samples for exposed and non-exposed participants, separately. The distributions of the participants' socio-demographics and other explanatory variables were explored, using the Chi-square test and parametric tests used to examine differences among the exposed and non-exposed participants (Antonio et al., 2013). The Chi-square test was used to examine the association between exposed and non-exposed participants with socio-demographics and other descriptive variables and regression analysis was conducted only for compounds detected in at least 15 of the study population (Afata et al., 2021, Freire et al., 2017). Finally, Factors associated with dependent variables (pesticide chemicals) were determined at a significant level of p-value < 0.25, were chosen in the bivariate, and included in regression models. The variables to be retained

in the model were then subjected to a stepwise backward elimination process based on p-values < 0.25. This process started with a complete model and eliminated each variable with the highest p-value one at a time until only variables with p-values < 0.05 were left for analysis.

## 8. Ethical consideration

The ethical approval to conduct this research must be obtained from your organization with a reference number and written informed consent from the study participants. All subjects participated in the study voluntarily.

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