A STUDY ON ORGANOPHOSPHATES PESTICIDES IN CHHATTISGARH

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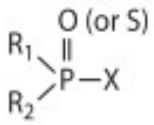
ABSTRACT

Organophosphorus pesticides are being most widely used as insecticides, and thus cause poisoning cases frequently in Chhattisgarh. The organophosphorus pesticides at the early stage, such as parathion and TEPP, had powerful insecticidal effects and high toxicity for both humans and beasts, and caused poisoning accidents during spraying. Although many less toxic organophosphorus pesticides were then developed, the resistance to the pesticides was acquired by insects during their repeated use, resulting in less effectiveness of the pesticides. Thus, after year and with the repeated application of organophosphate agents, many problems have appeared as a result of excessive use of pesticides. The adverse effects of pesticides are well documented in human health, environment, pesticide residue in crops, soil and water contaminated by these pesticides. Therefore, it is necessary to shed more light on the risks associated with the irresponsible usage of organophosphate pesticide

Keywords— Organophosphate, pesticide,human health and environment

INTRODUCTION

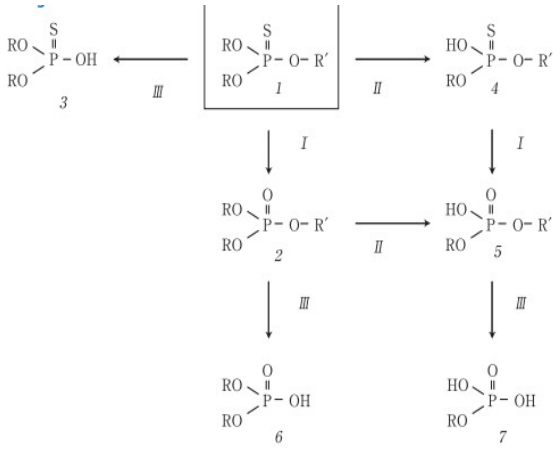
Chemical structure of organophosphorus pesticides is as below



The pesticides are also structurally classified according to an element bound with the phosphorus into the phosphate type, thiono type, thiol type and dithiol type. Many of R_1 and R_2 are dimethoxy or diethoxy groups. To cope with resistant insects, the alkyl moieties of R_1 and R_2 groups were replaced by unsymmetyrical propyl and ethyl groups, respectively, in some pesticides. As X structures, alkyl, alkoxy, alkylthio, aryl, heterocyclic, aryloxy and arylthio groups can be mentioned[1].

Organophosphorus pesticides undergo both enhancement of their toxicity and detoxification at the same time in mammals. The thiono type pesticide (1) shows almost no inhibitory action on cholinesterase in its unchanged form; it is metabolized by cytochrome P_{450} (I) into the phosphate type (2), which reveals toxicity. Therefore the phosphate type pesticide can inhibit cholinesterase without any metabolic activation[2].

A metabolic pathways for organophosphorus pesticides in mammalsas shown below-



An analytical instruments for organophosphorus pesticides, GC and HPLC are being used. Especially GC with detectors specific for phosphorus or sulfur is useful, because of its high sensitivity and specificity. The metabolites are usually highly polar, and thus need derivatizations for GC detection[3].

MATERIAL AND METHODS

Standard compounds: Generally We are used highly pure organophosphorus compounds can be obtained from Arexchemical ,Mumbai.

- Standard solutions: each standard compound is dissolved in acetone to prepare 1–10 µg/ mL solutions; these solutions can be further diluted according to needs. As an internal standard (IS), an organophosphorus compound, which does not overlap the peaks of a target compound and impurities, is chosen in preliminary experiments.
- Solid-phase extraction cartridges: Sep-Pak C18 cartridges.
- Methylating reagent: N-methyl-N-nitroso-p-toluenesulfonamide(Sigma-Aldrich); the distillation device attached with a 25-mL reservoir is constructedinside a draft ; the reservoir is immersed in an ice bath. A 0.4-g amount of KOH is dissolved in 10 mL of

ethanol/water (10:1,v/v)[1]

A.14-g amount of N-methyl-N-nitroso-p-toluenesulfonamide is dissolved in 30 mL diethyl ether (0.01 M) and stored in a 250-mL volume round-bottomed flask under ice-cooling; after 10 mL of the above KOH-ethanol solution is added to the above solution and mixed, the flask containing the mixture is connected with the distillation device and left at room temperature for several minutes. Then, the flask is warmed at 35–40 °C for distillation until the color of the solution in it disappears; this procedure results in collecting about 12 mL diazomethane-diethyl ether solution. If all solution is stored in an airtight container, there is a possibility of its explosion due to generation of N_2 gas. The solution can be stored in a flask with a drying tube at –20 °C. Another method is its storage at –20 °C aft er putting 2-mL aliquot each in 10-mL volume vials to be capped airtightly. While the ether solution is yellow, it can be used for methylation[3].

Organic solvents: All the organic solvent are used in ultra-pure grade.

A. **Solid-phase extraction**

i. Each Sep-Pak C_{18} cartridge is washed with 10 mL of chloroform/isopropanol (9:1), 10 mL acetonitrile, 10 mL of acetonitrile/distilled water (1:1) and 10 mL distilled water.

ii. A 1-mL volume of plasma or urine is diluted 10-fold with distilled water, and mixed with ISb; the mixture solution is poured into the above pretreated cartridge at a fl ow rate of 5 mL/min to trap target compounds including IS in the cartridge.

iii. The cartridge is washed with 10 mL distilled water, followed by the elution of the target compounds with 3 mL of chloroform/isopropanol (9:1). Th e eluate is collected in a glass vial. A small amount of aqueous upper layer is removed with a Pasteur pipette, and the organic layer is evaporated to dryness under a stream of nitrogen. Th e residue is dissolved in 100 µL acetonitrile, and a 1–2 µL aliquot of it is injected into GC(/MS) [1].

B Liquid-liquid extraction

i. A 1-mL volume of plasma or urine, 2 mL distilled water and IS are placed in a 10-mL volume glass centrifuge tube with a ground-in stopper; the pH of the mixture is adjusted to 2 with 1 M HCl solution. ii. Aft er adding 2 mL chloroform, the tube is vortex-mixed for 2 min and centrifuged at 3,000 rpm for 5 min to separate the chloroform phase; this procedure is repeated twice, and the resulting chloroform phases are combined and evaporated to dryness under a stream of nitrogen

ii. The residue is dissolved in 50–100 µL acetone; a 1–2 µL aliquot of it is injected into GC/ MS. iv. Methylation of metabolites: aft er measurements of the final acetone solution 2–3 times, the remaining acetone solution is evaporated to dryness; the residue is dissolved in 100 µL of the diazomethane-ether solution and left at room temperature for 5 minute. After evaporation of the solution to dryness under a stream of nitrogen, the residue is again dissolved in 50–100 µL acetone; 1–2 µL of it is injected into GC-MS). The obtained TIC is carefully compared with that before methylation. When a new peak appears or the amount of an organophosphorus pesticide is increased, there is a possibility of the presence of a metabolite.

ORGAN SPECIMENS

i. A 1–2 g amount of an organ specimen is excised and put in 4 volumes of acetonitrile; the organ tissue is minced into small pieces with surgical scissors and homogenized with a Polytron homogenizer (Kinematica, Luzern, Switzerland) or Ultra-disperser (Yamato, Tokyo, Japan) for 2 min for both extraction and deproteinization.

ii. The homogenate is centrifuged at 3,000 rpm for 5 min to separate supernatant solution; to the sediment the same amount of acetonitrile is added, vortex-mixed and centrifuged to obtain the 2nd supernatant solution

iii. Both supernatant solutions are combined and condensed to about 0.5 mL under reduced pressure with warming at not higher than 40 °C.

iv. To the condensed solution, 4.5 mL distilled water is added; when insoluble residues are present, they are removed by centrifugation. The clear supernatant solution is subjected to the above liquid-liquid extraction or solid-phase extraction[4].

RESULTSANALYSIS

Here we can use $Ce(SO_4)_2$ reacts with organophosphorus insecticides (malathion and dimethoate) with the consumption of 25 mol of $Ce(SO4)_2$ per each mole of organophosphorus insecticides giving a mixture of products[16]. The remaining Ce^{4+} reduces the color intensity of C_2R or Rh_6G through disruption of the conjugation system in the dye. NBS reacts with malathion and dimethoate with consumption of 5.0 mol of NBS per each mole of malathion and dimethoate giving a mixture of products. The remaining oxidant reduces the intensity of red color of AM dye through disruption of the conjugation system in the dye[5].

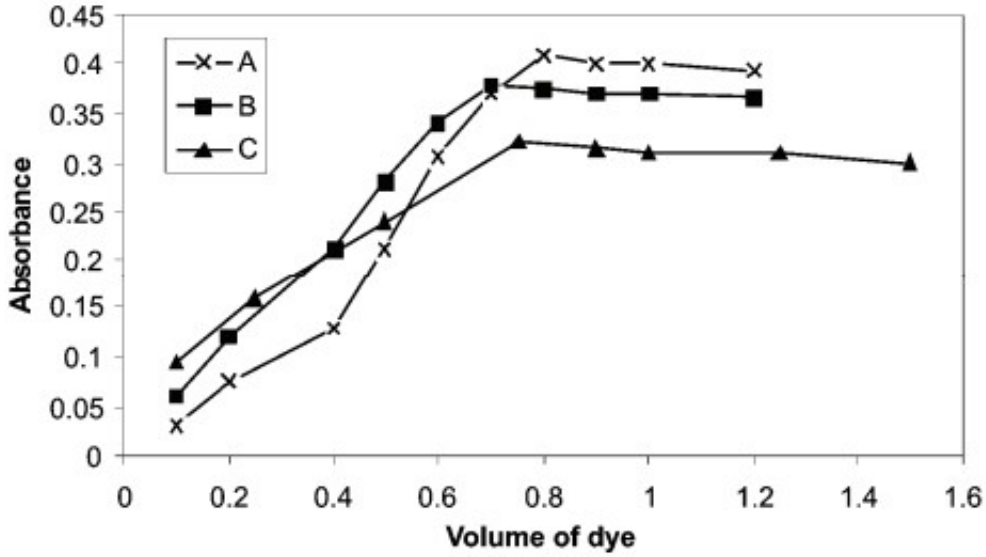


Fig1. Plot of graph between absorbance and volume of dye

The remaining colours stay constant in absorbance for at least 48 h, and then slightly decrease afterwardsSeveral trials were taken using ethyl acetate, methanol and acetonitrile as a dilution media. Absorbance of Chlorpyrifos and Prophenofos was found to be highest in ethyl acetate solvent compared with other solvents[6], hence, ethyl acetate was taken as a solvent. Between 250-400 nm, spectra of both the pesticides were noiseless in ethyl acetate. Zeroorder spectra[12] for both the pesticides having concentration 10 µg/mL were scanned in 200-400 nm range[7]. Zero order spectra showed the overlapping of band for both of the pesticides. So, first order derivative UV spectrophotometric method was performed . From spectral observation, 270nm 280 nm were selected as analytical wavelength for measuring the amplitude of chlorpyrifos and prophenofos, etc. In HPLC method:Suitable aliquots of standard stock solution of chlorpyrifos and prophenofos (200 µg/mL) were taken in separate 200 mL volumetric flasks and diluted with acetonitrile to obtain solutions containing chlorpyrifos and prophenofos having concentration in range of different ranges in µg/mL[8]. The samples were injected in HPLC system under optimized chromatographic conditions and chromatograms were recorded at approx. 220nm[9]. Calibration curves were constructed by plotting the peak area versus concentration and the regression equations were calculated for both the analyte. Each response was the average of six determinations.[10]

HEALTH IMPACTS OF ORGANOPHOSPHATE PESTICIDES

Health of human body organs like brain, liver, kidneys, ovaries etc., were affected by organophosphates poisons causing structural and chemical changes[19], as well as growth retardation. In a study carried out for two years with dogs, results showed that atrazine dose caused reduction in food intake and increased heart and liver weights[13].

Some data shown that on the effects of organophosphate occupational exposure on pregnant women and their fetuses are not available, although such information would be valuable. In humans, only a few cases of acute organophosphorus insecticide poisoning during pregnancy have been described. Continuation of the pregnancy was considered to be dangerous[14].

A number of pesticides clearly have the potential to cause reproductive toxicity in animals, and many compounds e.g. (chlordecone) are known to affect human reproduction. Also, OPPs including insecticides (malathion, parathion, diazinon, fenthion, dichlorvos, chlorpyrifos, ethion), nerve gases (soman, sarin, tabun, VX), ophthalmic agents (echothiophate, isoflurophate), and antihelmintics (trichlorfon). Herbicides (tribufos [DEF], merphos) are tricresyl phosphate containing industrial chemicals[18]. Cytogenetic damage related to pesticides exposure has been reported in various populations[15].

People exposed to pesticides are at increased risk of contracting certain cancers known to be associated with immune suppression[20]. In summary, pesticides could cause a variety of cancers through an immunological mechanism[17]. There are scientific evidences suggesting that many pesticides damage the immune system. Animal studies have shown that pesticides alter the immune system’s normal structure, disturb immune responses, and reduce animal’s resistance to antigens and infectious agents. In the case of Malathion which is considered a very low toxic compound[16].

CONCLUSION

A new spectrophotometric and HPLC methods are efficient for the determination of organophosphorus pesticides viz. chlorpyrifos and prophenofos and others also present in water, soil, fruits, rice urine and human blood and vegetables. It was found that the methods were accurate, precise, sensitive, robust and easy to apply. samples collected from different regions in the state of Chhattisgarh. This method is highly sensitivity, simplicity, reproducibility, precision, accuracy and stability of colored specie. The pesticide residues are present in higher amount in

the different outdoor sample viz. water, soil, fruits, rice urine and human blood and that we are consuming.

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