Comparison of ELISA and GC Methods to Detect DDT Residues in Water Samples

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ELISA and GC methods were used to analyse DDT residues in about 30 water samples collected from different taluks of Mandya District of Karnataka. Polyclonal antibody based immunoassay developed at CFTRI, Mysore, performed well to detect the DDT residues. The minimum detectable level of DDT by ELISA was one part per billion (ppb) in the water samples tested. The insecticide residue ranged from 1 to 20 ppb. Experiments also revealed no matrix effect and hence did not require any prior clean-up. The pH of the water did not interfere in the assay. The ELISA method validated in the present work is specific to DDT. The results of ELISA with respect to DDT residues were found to be comparable to values obtained from the GC analysis of the water samples. The water samples could be directly used for ELISA test, thereby making the analysis quick, simple and cost effective.

Keywords: DDT residues, ELISA, GC, water samples

Introduction

DDT [1,1′-(2,2,2-trichloro diphenyl trichloro-ethylen e)] has been extensively studied for its toxicity. Monitoring of ubiquitous environmental pollutants such as DDT, has always been considered important for controlling human exposure. As compared to several other countries, the higher body burden of pesticides in Indian population and in drinking water is indicative of higher exposure to these chemicals (Jani et al., 1998). India is one of the major point source countries in the tropical belt and a reason for the global contamination by organochlorines. DDT constituted a major portion of the total pesticide use in India (Gupta, 1991). Pesticide pollutants are hydrophobic in nature, get adsorbed on soil/sediment and accumulate in the tissues of organisms. Bottom sediments and soil is the ultimate sink for pesticide pollutants, which are brought into aquatic system through surface run-off and atmospheric fall-out.

In India, DDT was routinely sprayed to control mosquitoes to eradicate malaria in most of the irrigated lands growing rice and sugarcane. There are chances for DDT to contaminate the different water sources.

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DDT is generally analysed by gas chromatography (GC) with electron capture detector, mass metry or High Performance Liquid Chromatography (HPLC) (Dikshit et al., 1990). Each of these methods, which needs pesticide extraction, clean-up and concentration of the extract by well-trained personnel, is labour intensive, time-consuming and expensive, making the method unsuitable in environmental studies and export sample analysis. With the increasing demand for pesticide residue analysis certification in export houses, quarantine department and textile trade industries, there is a need to develop simple, quick, cost effective and sensitive assays to detect pesticide residues. Under these circumstances, immunoassays are fast being developed and becoming popular.

Immunoassays are now routinely used in clinical analysis of proteins, hormones and drugs, and developed both as screening tools and as quantitative analytical methods for pesticide residues in the environment (Jung et al., 1989; Wratten & Feng, 1990; Ferguson et al., 1993; Skerritt, 1994; Skerritt & Amitarani, 1996; Karanth et al., 1998). ELISA is fast gaining ground as one of the quick analytical methods for analysis/screening of pesticide residues in foods, soil, water etc.

Considering the use of DDT in India for the eradication of malaria, the present study was undertaken to monitor DDT in water samples from...
different sources in a highly irrigated agricultural district. The study covers the effect of the matrices in the water samples on the DDT assay and validates the ELISA data with GC analysis. This is first report regarding comparative study of ELISA (competitive) and GC as a detection method for residues of DDT in different sources of water samples.

Materials and Methods

Materials

The general glasswares and other plastic wares, required for analyzing DDT in the samples by ELISA and GC, were procured from the local market. Chemicals. All the fine chemicals were procured from Sigma, USA. General solvents and salts of high purity were obtained locally. High binding immunoassay plates were purchased from Nunc, Denmark. The Hapten, polyclonal antibody and HRP-pesticide conjugate for DDT assay were produced at CFTRI, Mysore using New Zealand rabbits. The rabbits were procured from the Animal House, CFTRI and maintained under good laboratory conditions at the Central Animal Facility, CFTRI. The rabbits were fed pellets and water ad libitum.

Water samples. About 5-10 water samples were collected from different areas from Mandya District, Karnataka, amounting to a total of 30 samples. The samples were collected from the following areas: Srirangapatna (Samples S1 to S10), Nagamangala (N1 to N3), K.R. Pet (K1 to K3), Pandavapura (P1 to P5) and Malavalli (M1). The water samples were from sources like borewell, lake and channels. The water from these sources are also used for drinking purpose in and around Mandya District, Karnataka, which is about 40-50 kms away from the host institute. The samples were stored at 4°C before use and analyzed within 24 hrs of collection.

Methods

1. Hapten design and synthesis. The Hapten used in the present study was DDT-OH conjugated to ovalbumin. The details of the synthesis and design have been described in Amitarani et al (2001).

2. Antibody production. Rabbits were used for the production of antibody according to the method described in Amitarani et al (2001).

3. Pesticide dilution. DDT was dissolved in methanol to get a 1000-ppm solution (1mg/ml). It was serially diluted with distilled water from a 1000 ppm stock to get final concentrations of 10, 3.3, 0.3, 0.1, 0.03, 0.01 and 0 ppm. Pesticide dilutions were made using solvents, which were further diluted by 1/10 in phosphate buffered saline with 0.5% fish gelatin (PBS-FG) before loading onto the plate. Pesticide dilution must be done in glass tubes as pesticides stick to plastic surfaces. Each pesticide dilution from 0 to 10 ppm was added in duplicate to the antibody-coated plate. The antibody was coated in carbonate buffer (pH 9.6) at a concentration of 1 μg/100 μl/well and left overnight at room temperature. The plate was emptied the next day and washed with buffer (pH 7) and blocked with 1% BSA solution (150 μl) and left for 1 hr incubation at room temperature. The BSA solution was then removed and the plate tapped dry on a towel/tissue paper and used to perform the assay.

4. HRP-pesticide conjugate dilution. It was prepared by dissolving the HRP in phosphate buffer, cooled in an ice bath and the active ester dissolved in dry DMF, shaken and allowed to sit at 8°C overnight, dialysed three times against PBS and stored at 8°C. Equal quantities of HRP and Hapten were conjugated in distilled water in the presence of EDC, dialysed against PBS and used. HRP conjugate was diluted in 50 mM PBS-FG. A stock of 1/2 k was made in 50 mM PBS-FG. 100 μl of this 1/2 k stock was added to the 15 ml of 50 mM PBS-FG to get final working concentration of 1/300k.

Procedure to Load the Plate

Each 100 μl of pesticide and HRP-conjugate (1/300k) were added in duplicate to the plate wells. The pesticide and HRP were allowed to bind with the antibody for 1 hr at room temperature. The plate was then washed with wash buffer three times to remove the excess pesticides/HRP and the plate tapped dry. Then the substrate chromogen (tetramethyl benzidine) solution was added (150 μl) and allowed for 30 min for the colour development. The reaction was stopped after 30 min by adding 50 μl of the stop solution (2.5N H2SO4). The absorbance was read at 450 nm on a plate reader. A standard graph was plotted using the OD vs Concentration on a semi log graph.

In this method, greater the concentration of the pesticide, lesser the colour is produced at the end of the test.

Matrix Effect Studies

Organic solvents are used to extract the pesticides from the food sample. Often, along with the pesticide,
various constituents of the food/water like carbohydrates, pigments, fats, mineral salts are also extracted. These constituents are called matrices and can interfere in the ELISA. Thus, there is need to remove such matrices from the samples before performing the assay.

Matrix effect of water on DDT assay. The water samples to be analysed were used directly in the preparation of a matrix curve and a graph was prepared where 1000 ppm pesticide solution was diluted with the samples instead of distilled water. If there is no matrix interference, the matrix curve will run parallel to the standard curve with distilled water. In the presence of matrix interference, the curve shifts to the right.

Analysis of DDT Residues in Water Samples by Immunoassay

The samples stored at 4°C were analyzed within 24 hrs of collection. 100 μl of water sample was added directly to the well and concentration of DDT in these samples was read against the distilled water standard graph (positive control).

Analysis of DDT Residues in Water Samples by GC

The samples were analysed by a modified method (Dikshit et al, 1990). In a separating flask, 5 ml of water sample was taken. To this, 5 ml of hexane was added. The flask was shaken well to shift the pesticide from the water sample to the hexane layer, which was collected in the test tube and allowed to evaporate to dryness. The dried residue was stored at 4°C. The residue was dissolved in 20 μl of hexane and 1 μl of the sample injected. The pesticide extraction was according to the method of EPA.

Estimation of DDT Using GC

DDT was estimated using GC (GC 8000 series Fisons instruments) equipped with a 35N electron capture detector connected to a digital venturias data processor. A 183 cm x 5.1 cm stainless steel column packed with 10% OV-17 (non polar vinyl silicon) on chromosorb-W was used and the temperatures were kept as: injector, 250°C; column: 230°C; and detector, 300°C. The carrier gas was N₂ (99.9% pure) with a flow rate of 30-ml/min. DDE and DDT standards were dissolved separately in GLC grade hexane and different concentrations were injected. A standard graph was prepared by plotting DDT and DDE concentrations against the square root of the peak areas.

Estimation of DDT and DDE in the Water Samples by GC

The cleaned and dried residues from water samples of different places were dissolved in hexane (20 μl), of which 1 μl was injected into the GC at the standard conditions described above for resolving DDT and DDE. This method permitted the estimation of DDT and its metabolite DDE simultaneously without prior separation of the two from the extracted mixture.

Quantification

Peak areas and retention time of DDT and its metabolite were compared with respective standards injected after every 3 samples.

Results

I. Standard Graph of DDT by ELISA

In the DDT standard graph by ELISA (Fig. 1), the IC₅₀ value was 10 ppb. In the competitive assay, absorbance is inversely proportional to the pesticide concentration.

II. Matrix Effect on DDT Assay

On analyzing different samples by ELISA, matrix effect was seen only in two muddy samples (Fig. 2). The pH of the samples ranged from 6-8 and had no adverse effect on the assay.

III. Analysis of DDT Residues in Water Samples

(a) By ELISA. ELISA could detect DDT residues in

![Fig. 1—Standard graph of DDT by ELISA](image)
water samples. The minimum residue detected was 1 ppb and the maximum was 20 ppb in the samples.

(b) Standard graph of DDT by GC. DDT and DDE, as shown in the standard graph (Fig. 3), could be resolved easily and had a retention time of 19.47 and 13.2 min, respectively.

Estimation of DDT and Its Metabolite

The GC-ECD analysis showed that the least detectable quantity was 500 pg for both the molecules under the above explained conditions of analysis and the R, for DDT and DDE were 20th and 15th min, respectively. The relationship between the concentration and square root of the peak area was found to be linear. The concentration of DDT and DDE residues are shown in Fig. 4.

Comparison of GC and ELISA as Analytical Tools for Pesticide Residues in Water

GC and ELISA were comparable (Fig. 5) having good correlation, r= 0.98.

Discussion

The water samples collected for this study from different sources are used by the local people for drinking, cattle rearing and irrigation. The residues found in the different water samples were DDT (1-20 ppb) and DDE (1-6 ppb). The detected DDT and DDE residues were within the minimum daily intake range in water (Anonymous, 1986). Thus, it can be concluded that the water is safe for consumption and does not have any adverse effect if consumed/used for various activities.
Fig. 3—Standard graph of DDT and DDE on GC

Fig. 4—DDT and DDE residues as analysed by GC in the collected water samples
The present study indicates that analysis by ELISA is comparable to GC and can be used for the analysis of DDT residues in water samples. ELISA has more advantage over GC in that the sample could be used directly without clean-up. By ELISA, all the 30 samples could be analysed within two days, whereas by GC, the whole process takes about 15 days. The DDT residues found were within the permissible limits and were fit for drinking and other uses.

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References


