

## WATER SAMPLING AND SAMPLE TREATMENT

### Materials

All the reagents and solvents used were of analytical or chromatographic grade. Eighty (80) different pesticide standards ordered from Dr. Ehrenstorfer GmbH, (Ausburg, Germany) were used. Working standard solutions were made by dilution of these stock standards and mixtures of standards of different concentrations were used in most cases for the screening of the pesticide residues. All glassware used had teflon-stoppers.

### Sampling

Water samples were collected from few centimeters below the surface into 1-litre Teflon capped glass bottles. Sampling was done using a water sampler in the rivers running through the cotton fields and some selected points on the shores of lake Victoria. Duplicate samples were taken, one for determination of physico-chemical parameters and one for analysis of pesticides. The physical parameters; pH, temperature and conductivity were determined on site. Preservation was done in the field by adding 10% NaCl and shaking to proper mixing. The samples were kept at  $< 4$  °C until extraction.

### Extraction

Unfiltered water samples, previously preserved with 10% NaCl, were extracted by Liquid-Liquid Extraction (LLE) method (Åkerblom 1995). Each sample (1 l) was quantitatively transferred to a one litre-separating funnel and the bottle rinsed with dichloromethane (30 ml), and combined with the sample in the separating funnel. The combined contents were then successively extracted with dichloromethane (3 x 50 ml). The organic layer was filtered through a plug of glass wool containing anhydrous sodium sulphate (*ca* 30g) for drying. The sodium sulphate was later rinsed with dichloromethane (2 x 3 ml), the combined extract concentrated *in vacuo* at 30 °C, and the solvent changed to cyclohexane. The volume was adjusted in a stream of air to 2 ml in 9:1 cyclohexane: acetone (v/v) ready for GC analysis. The water extracts appeared clean and were not subjected to further clean up. However, on injection into GC, the peaks for dieldrin and DDE-p,p' in the SE 30 column may overlap, and hence to remove any ambiguity, sulphuric acid treatment was employed to destroy dieldrin. When sample extracts were found to be visibly dirty, they were cleaned up by GPC.

### *Clean up*

#### **Gel Permeation Chromatography (GPC)**

GPC employs a size exclusion principle in cleaning up different sample extracts for pesticide determination. It is routinely applied in most pesticide laboratories due to its versatility and applicability to a wide range of matrices and hundreds of pesticides.

A commonly used GPC uses a glass column of internal diameter 1 cm with one adapter in either end. A six-way valve is used to control the solvent through or past the column, and through or past a loop for loading and injecting the sample.

The stationary phase mostly used is Bio-Beads S-X3 with a mixture of cyclohexane/ethyl acetate or cyclohexane/dichloromethane both in the ratio 1:1 (v/v) as a mobile phase. The compounds are separated mainly according to their differences in molecular weights, although hydrophobic interactions are also involved due to the nature of the stationary phase.

Pumps have been commonly used to force the solvent through the column and sometimes gravity is used instead, by positioning the solvent reservoir a few metres above the column. The GPC device needs a frequent calibration to set a breaking point (the point at which pesticides starts to elute), otherwise one may lose the pesticide fraction or collect impure fractions. The pesticide fraction is among the compounds of molecular weights ranging between 200-500, which are normally eluted within 20 ml of solvent after the break point.<sup>1</sup>

### **Operation of the GPC device**

Before sample injection the valve is set to load position, thus blocking any solvent flowing through the loop. The loop is then cleaned thoroughly with acetone, ethanol and the respective eluent mixture successively. The sample is carefully introduced by using a syringe to fill the loop, thereafter, the loop is set at inject position to allow an even solvent flow through the whole system. Collection of the first fraction (the heavier molecules) starts immediately as the system starts and ends up at the break point. A fraction of 20 ml collected after the break point is expected to contain all the pesticides, and thereafter, 20 ml are discarded before introducing another sample for clean-up. The 20 ml pesticide fraction is then concentrated to appropriate volume and the solvent changed to 9:1 cyclohexane/acetone for GC analysis.

### **Analysis and quantification**

Analysis for the residues and metabolites was done as described by Åkerblom (1995). Varian Star 3400 and Hewlett Packard 5890A gas chromatographs equipped with <sup>63</sup>Ni Electron Capture (EC) and Nitrogen-Phosphorous (NP) detectors were used for the analysis. SE-30 and OV-1701 megabore columns (30 m x 0.32 mm x 0.5 µm) were used in each detector. Nitrogen was used as both a carrier and make up gas in the ECD at a flow rate of 30 ± 1 ml/min. In the NPD, helium was used as a carrier gas at a flow rate of 0.5 - 1 ml/min and nitrogen, at a flow rate of 29 ± 1 ml/min, was used as the make up gas. The temperature programme was 90 °C held for 1 min, 30 °C/min to 180 °C, 4 °C/min to 260 °C held for 12 minutes. The injector and detector temperatures were 250 °C and 300 °C, respectively. Identification of residues was effected by running samples and external reference standards in GC and then comparing the chromatograms. A peak was not considered relevant unless it appeared in both columns in a given detector.

### **Analysis and quantification**

Analysis for the residues and metabolites was done as described by Åkerblom (1995). Varian Star 3400 and Hewlett Packard 5890A gas chromatographs equipped with <sup>63</sup>Ni Electron Capture (EC) and Nitrogen-Phosphorous (NP) detectors were used for the analysis. SE-30 and OV-1701 megabore columns (30 m x 0.32 mm x 0.5 µm) were used in each detector. Nitrogen was used as

both a carrier and make up gas in the ECD at a flow rate of  $30 \pm 1$  ml/min. In the NPD, helium was used as a carrier gas at a flow rate of 0.5 - 1 ml/min and nitrogen, at a flow rate of  $29 \pm 1$  ml/min, was used as the make up gas. The temperature programme was 90 °C held for 1 min, 30 °C/min to 180 °C, 4 °C/min to 260 °C held for 12 minutes. The injector and detector temperatures were 250 °C and 300 °C, respectively. Identification of residues was effected by running samples and external reference standards in GC and then comparing the chromatograms. A peak was not considered relevant unless it appeared in both columns in a given detector. The method average detection limits are given in Table 1.

**Table 1 Average method detection limits**

Analyte	Sediments [(mg/kg dry weight) x10 <sup>-2</sup> ]	Water (□g/l)
$\alpha$ -HCH	3	0.4
$\beta$ -HCH	2	0.4
$\gamma$ -HCH	0.1	0.1
$\delta$ -HCH	0.1	0.2
<i>p,p'</i> -DDE	0.06	0.1
<i>p,p'</i> -DDD	0.7	0.3
<i>o,p'</i> -DDT	2	0.6
<i>p,p'</i> -DDT	10	0.6

Recoveries of residues were in the range 59 – 80 % for HCH and 70 – 99 % for DDT, which is within the acceptable range (Åkerblom, 1995).