The Freshwater Biological Association

ANALYSIS AND ADSORPTION
OF PHOSALONE
ON AQUATIC SEDIMENT

28.03.88 - 19.08.88

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1.0 INTRODUCTION

The Freshwater Biological Association is a non profit-making independent organisation founded in 1929 to pursue fundemental research into all aspects of freshwater biology. It has a membership of about 2000 including private individuals (biologists, naturalists, anglers), water authority undertakings, fishing clubs and universities. Most of its income, however, is a grant-in-aid from the Natural Environmental Research Council (NERC). It also receives substantial support from the Department of the Environment and the Ministry of Agriculture, Fisheries and Food who commision research projects relevant to their interests and responsibilities.

The Association has two laboratories,the headquarters of which are at the Windemere Laboratory which has a staff of 74 who are largely concerned with studies on the nearby lakes.The River Laboratory, situated at East Stoke, Dorset (fig.1), where the research project I was involved with was based, has a current staff of 23 who are involved mainly on the productive streams of the area.

The River Laboratory was formally opened in 1965 to provide a centre for the study of the relatively unpolluted and biologically productive rivers of central southern England. The site itself was specifically chosen to include a head of water in the Mill Stream which could supply experimental channels and provide flow to a fluvarium. Such facilities made the site ideal as a research facility which simulated the natural conditions of a river. Many experiments, however, require spring water which has not been affected by passage down a river and this is available at the Waterston Experimental Station, near Puddletown, where another range of experimental channels have been established. The Association also owns the fishing rights to almost 5 km of the River Frome and has access to many other sites on various rivers.

Management of the River Laboratory is undertaken by the Officer-in-Charge assisted by the various heads of departments and principal scientific officers. Staff positions at the River Laboratory comply with the normal civil service grading system.

Due to Government imposed financial restraints, there has been recent proposals by the Natural Environment Research Council to disband the River Laboratory and sell the site. Given, that the unique site represents an enormous financial and scientific investment which could not be regained in the foreseeable future, the staff are making a concerted effort to fight and counteract the proposals. It is to be hoped that their determined effort succeeds.

2.0 RESEARCH REPORT

2.1 Introduction

Following the 1987 report on pesticide spray-drift submitted to the Nature Conservancy Council (NCC/NERC CONTRACT HF3/02/208/4) which indicated the possible risk to benthic invertebrates due to the sorption of lypophilic compounds onto suspended and sediment particles, Dr W.A House and his colleagues were commissioned to ascertain which compounds were of particular danger and required further study. The available information on insecticides and mollusicides over the last few years were examined in detail (Chapman and Long, 1985 and 1986). From the review it was established that in terms of treated area the most used insecticides were the organophoshates and carbamates (Table 1). Further analysis of these compounds found that the most applied insecticides are phosalone, dimethoate, pirimicarb and demeton-S-methyl. The pyrethroids, permethrin and cypermethrin, although particularly toxic are less used. The results are summarised in Table 2. According to their solubility in water the effect of these insecticides on benthic invertebrates may be estimated. Working from the premise that the less soluble an insecticide is in water, then the more likely is it to be adsorbed onto sediments, it was decided that given the low solubility of phosalone and permethrin e.g. 2.15-10 mg/l and 0.02-0.07 mg/l respectively, then further study should be conducted on them. Table 3 lists the solubilities of the other selected pesticides.

Having selected phosalone and permethrin for study, it was decided to begin work on the latter first, as more information was available for the analytical determination of permethrin than for phosalone, although determination methods for sediment analysis was unavailable for either. The aim for both compounds was to establish a method for their determination in aquatic sediment, so that areas where they were sprayed could then be analysed to ascertain the concentration and effect of these compounds in the natural aquatic environment on the benthic invertebrates.

After initiating work on permethrin, it was decided to employ a sandwich student to begin the work on phosalone. As a student, I was employed for approximately 5 months to study phosalone. The work involved the following aspects :

1. A full literature review on all aspects of phosalone including :-
   a. Its physical, biological and chemical characteristics.
   b. Analytical methods of analysis with particular reference to soils/sediments.

2. To develop a method for the determintion of phosalone in aquatic sediments.

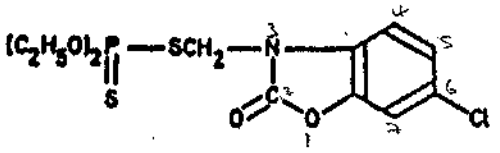
3. To determine the adsorption of phosalone onto kaolinite.

2.2 Literature Survey

2.2.1 General

Phosalone is a non systematic, wide spectrum organophosphate pesticide which was discovered in 1961 in the laboratories of the Societe des Usines Chimique Rhone-Poulenc in France. It has been approved for commercial use since 1964 in France, in Australia since 1966, in the United Kingdom in 1967 and in many other countries including Japan, Egypt, USSR and the USA. It was first reported by Desmoras, Lacroix and Metiever in 1963.

Phosalone has the following chemical structure and empirical formulae:



S-6-chloro-2,3-dihydro-2-oxobenzoxazol-3-yl O,O-diethyl phosphorodithioate (IUPAC)

S-((6-chloro-2-oxo-3(2H)-benzoxazoyl)methyl) O,O-diethyl phosphorodithioate (CA)

O,O-diethyl-S-((6-chloro-1,3-benzoxazol-2(3H)-onyl)methyl phosphorodithioate

Phosalone may also be found under a variety of manufacturer/product names:

Zolone, RP11974 Zolone, TAXY-Zolone, Zolone flo, Rubitox, Azofene, NPH-1091, RP-11974

It is formulated as wettable powders, emulsifiable concentrates and dusts, either alone or in association with other pesticides.

Phosalone is synthesisded as follows: Benzoxazolone obtained from urea and O-aminophenol, on chlorination gives 6-chlorobenzoxazolone. This is chloromethylated to 3-chloromethyl-6-chlorobenzoxazolone, which condenses with sodium O,O-diethylphosphorodithioate to yield phosalone.

2.2.2 Physical Properties

Phosalone is a stable, white, nonhygroscopic crystalline solid with a slight odour of garlic. It has a melting point of 45-48°C (Colinese and Terry 1968, Freed et al 1979), with a negligible vapour pressure at 20°C (Desmoras et al 1974). A discrepancy was found for values of phosalone solubility between 20 and 25°C, Freed et al 1979 evaluated it to be 2.15 mg/l while Desmoras et al 1974 determined it to be 10.0 mg/l. Colinese and Terry 1968 describe it as insoluble in water. The method used by Freed et al 1979 was evaporation of an ether solution of phosalone, followed by addition of distilled water then magnetic stirring and testing of concentration until values lay within 5% variability over a 5 day period. No method is presented by Desmoras et al 1974 or Colinese and Terry 1979 to explain the values that the obtained. Phosalone is readily soluble in ketones (1000 gm/l), alcohols (200gm/l) and in most aromatic solvents. (Desmoras et al 1974).

2.2.3 Biological Properties

2.2.3.1 Insecticidal and Acaricidal Activity

Phosalone is both an insecticide and an acaricide, the outstanding property of which is its broad area of activity. It is very active on all important mites and small insects (Homoptera, Heteroptera, Orthoptera, Lepideptera, Coleoptera and Diptera. Desmoras et al 1974). Its use is recommended in many countries for the treatment of various crops, in the United Kingdom it is applied to various cereals, field crops and peas/beans. The UK Pesticide Guide 1988 describes which crops phosalone is applied to and the and the insects which it is effective against, Table 4 contains a list of these.

2.2.3.2 Toxicity

Phosalone toxicity arises as a result of it being an inhibitor of the enzyme cholinesterase and is dependant on the concentration present and the organism with which it has come into contact with. Considerable study has been made about its toxicity, Table 5 contains a summary of the LD50 values (where applicable). Twinn and Lacy 1979 describe phosalone as non toxic to bees at concentration of 462 and 660 g/ha providing they are not actively foraging at the time of application. Ambrosi et al 1978 found that in an experimental model aquatic ecosystem although the fish died the phosalone had no effect on water snails and algae growth was unaffected. Colinese and Terry 1968 found that phosalone was relatively non-toxic to game birds.

2.2.4 Chemical Properties

2.2.4.1 General

Phosalone is a non-corrosive organophosporous compound which is relatively stable to heat (upto 100°C). It is hydrolysed in solution by stong acids and alkalis. Ambrosi et al 1977 found the time it takes for phosalone to degrade to half its original concentration (its half-life) in Matapeake and Monmouth fine sandy loam to be between 3 and 7 days. Its half-life was determined to lie within 6 to 8 days in cauliflower (Duhra and Hameed 1983), 1.02 days in tomatoes (Rajukkanu et al 1983) and 1.018 days in brinjal friut (Rao et al 1986). Phosalone is, however, fairly persistent in treated plants with upto 40% found in the plant tissues after 15 days (Colinese and Terry 1968). Phosalone has a octanol-water partition co-efficient of 20,000 (log P = 4.30) (Freed et al 1979). No information was attainable on other the important characteristics which are considered useful in assessing the environmental impact of insecticides in the aquatic systems (photoreaction constant, volatilisation rate constant, and the bioconcentration factor).

2.2.4.2 Metabolism in plants

Desmoras et al 1963, 1967 found that phosalone-oxon is formed in treated plants but it is degraded much more rapidly than phosalone, so that the amount present is usually only about 1% and never more than 10% of the phosalone residue. Leffingwell et al 1977, however, found in their study of dislodgeable zolone residues in commercially treated vineyards, that the degradation of the oxon did not overtake its formation from the parent compound and did not show signs of doing so even after 35 days post application. Continuing their work on phosalone metabolism Demoras and co-workers found that the first point of hydrolytic attack on phosalone, or the phosalone-oxon occured at the P-S-C link giving O,O-diethyl phosphorodithioic acid and O,O-diethyl phosphprothioic acid and a benzoxalone moiety. In plants the latter is matabolised further to give a glycoside. Figure 2 illustrates this process graphically (Desmoras et al 1967. Colinese and Terry 1968)

2.2.4.3 Degadation in soil

The degradation of soil was also examined by Desmoras et al 1967. They found that the first stages followed those in plants but a red material was obtained on degradation of the benzoxazolone moiety giving a substituted phenoxazone. An additional fragment containing phospherous was shown to be a disulphide formed by oxidation of the phosphorodithioate. Figure 3 illustrates the degradation in soil.

2.2.4.4 Microbial degradation

Golovleva et al 1983 studied the degradation of phosalone by the soil microorganisms. The cultures, able to utilise the phosalone as a source of carbon and phospherous were isolated. Pathways and conditions of degradation by Acinetobacter calcoaceticus 27f were selected for further study. It was found that the conditions of aeration had no principle influence on the degree of the phosalone degradation. The reactions of hydrolysis were found to also be the initial stages of the insecticide transformation. Phosalone is degraded to 2-amino-7-chlor-3H-phenoxasin-3-on via condensation of 2-amino-7-chlor-3H-phenol. The former accumulates in the culture liquid and soil for 3 months or more. Figure 4 illustrates the pathway.

2.2.5 Analytical Methods for Determination

2.2.5.1 General

In reviewing methods for the analytical determination of phosalone, primary interest was focused on Gas Chromatographic (GC) methods, principally because the majority of pesticide analyses are carried out this way. In addition on-going work on permethrin utilised a Packard model 438 gas chromatogram with Electron Capture Detector (ECD). it was hoped that phosalone could also be determined similarly, preferably using the same column.

Having decided on a GC method for phosalone analysis, further information was required on the procedures for (a) extraction from aquatic sediments, (b) extract cleanup and (c) GC determination.

2.2.5.2 Published methods

The literature search revealed no information on phosalone determination in aquatic sediments and very little on soils. Some information was, however, available on analysis of various crops, which could be applicable for sediments.

Desmoras et al 1974 recommended various extraction procedures, dependant on the material to be analysed (plants, soil, milk, meat, fatty crops and oils, and citrus crops). For soil, they advised acetone extraction by blending, in the prescence of anhydrous sodium sulphate, followed by solvent evaporation. Table 6 lists the recommended extraction techniques according to the sample material. As for soil, acetone extraction is advised for most materials.
Sample cleanup consisted of two steps in the Desmoras method, partition cleanup and column chromatography. For soil, crops and milk the partitioning phase was between water and dichloromethane, and between hexane and dichloromethane for the other materials. The extracts obtained were then evaporated to dryness before column chromatography. This step involved dissolving the residue in benzene (5 * 5 ml portions), combining the extracts and subjecting them to florisil chromatography on a 2.5 * 50 cm column containing 25 g of florisil. Elution of the column was succesive with 100 ml of benzene, then 300 ml of dichloromethane-ethyl acetate (98:2 v/v) or benzene-ethylacetate (95:5 v/v). The fraction obtained is then evaporated to dryness and made up to a known volume in acetone, ready for GLC determination.
GLC was the same for all the residues. The operating parameters for the two GLC models with ECD, using three different columns are described in Table 7. Desmoras suggests that they may be adapted to the available chromatogram. The actual detection limit of the analysis is dependant on the nature of the material and the efficiency of cleanup. For these methods, a detection limit of between 0.01-0.03 ppm was suggested.

2.2.5.2 Published Methods cont.

Ambrosi et al 1977 in a study of the persistence and metabolism of 14-carbon labelled phosalone in two types of soil (Matapeake and Monmouth fine sandy loam), extracted 10-20 gm core samples on a a rotary shaker with 50 ml of benzene-ethylacetate-acetone (1:1:2, v/v/v) and again in 10% aqueous methanol, each for 20 hours. Their method of determination was by Thin Layer Chromatography (TLC) and scintillation counters. In a subsequent project, Ambrosi et al 1978 studied the distribution of oxadiazon and phosalone in an aquatic model ecosystem, using 14-carbon labelling. Water samples were extracted using methylene chloride. Extracts were again analysed by TLC and scintillation methods. In neither study was extract cleanup required.

Kawamura et al 1978 presented a modified method for the simultaneous determination of 33 organophospherous pesticides, including phosalone, in water and 7 crops (cabbage, chinese cabbage, tomato, carrot, apple, japanese pear and mandarine orange). In their method, extraction by acetonitrile (2 * 100 ml) was followed by a second extraction with 20% dichloromethane in benzene (2 * 100 ml). The extract was washed with water (2*100ml) and dried over anhydrous sodium sulphate , then evaporated to 5 ml before cleanup. Using this method 87.5% of phosalone was recovered compared to 74.5% when a mixture of acetone and benzene was used in place of acetonitrile.
Extract cleanup was achieved using charcoal and anhydrous sodium sulphate in a column. The column was eluted before and after addition of the phosalone extract. The latter elulate was collected (150ml) and evaporated to dryness before being concentrated in 2 ml of acetone. For phosalone, elution with a mixture of 50 % acetonitrile in benzene actually gave a better recovery (100%) than by using benzene on its own. Benzene was, however, the most efficient solvent for the simultaneous cleanup.
Using a Gas Liquid Chromatograph, model 4BMPF with a flame photometric detector, analysis of the acetone residue yielded recoveries of phosalone in the crops, ranging from 80.0 to 100.0 %. Recovery from water was 98.5%.

V.R. Rao et al 1986 in a study of the persistence of fenithrothion and phosalone in brinjal (fruit), extracted random samples three times, first with 100ml and subsequently with 50 ml of redistilled n-hexane and acetone. The extracts were filtered and concentrated, then passed through a glass column containing a mixture of activated charcoal-celite-magnesia (2:1:1) followed by elution of the column with a hexane and acetone mixture until about 500 ml of elulate was collected. Following drying with anhydrous sodium sulphate the extract was concentrated to 10 ml with it is presumed the same eluting solvent.
Determination of the cleaned up extract was by a pyridine method.

2.2.5.2. Published Methods cont.

A similar method is employed by B.N. Rao et al 1986 in the analysis of phosalone in tomatoes, as is used by V.R. Rao et al 1986. The only differences are in the column used for cleanup, where in this case the ratio of celite, magnesium oxide and charcoal is 1:1:1. Using this method, the phosalone extracts were seperated by solvent partition technique.

Duhra and Hameed 1983, in a study of the toxicity of 5 field-weathered insecticides to the cabbage butterfly, present a biological (bioassay with Drosphilia melanogaster) and a chemical (GLC) method for the simultaneous determination of the insecticides (including phosalone) in cauliflower. Extraction was carried out using a Toshniwal model RL 04/01A was used to verify the biological method. The GLC configurations are described in Table 7. Both methods gave a recovery for all the insecticides ranging from 86.0 to 90.55%.

2.2.6 Discussion

The literature survey revealed relatively little information on phosalone, aside from its general physical, biological and chemical characteristics. No information was found on phosalone determination in aquatic sediments although some was available for soils (Desmoras et al 1971. Ambrosi et al 1977), and crops (Kawamura et al 1978, V.R. Rao et al 1986, B.N. Rao et al 1986, Duhra and Hameed 1983).

2.3 Analytical Methods for Determination of Phosalone in Aquatic Sediments.

2.3.1 Introduction

Using the published methods for determination of phosalone in soils and crops, the basis of a method for analysis in aquatic sediments had to be decided upon. That chosen for development in this project involved gas chromatography with ECD, sample cleanup on an adsorption column and solvent extraction from sediment.

2.3.2 GC Determination

2.3.2.1 Introduction

On-going work on permethrin at the river laboratory utilised a Packard model 438 Gas Chromatogram with ECD and a 1m * 4mm I.D glass column packed with 2% SP2330 on Chromosorb W-HP (100-120 mesh) This column was a recommended type for Permethrin analysis, however, an alternative packing which could have been used was 5% OV-210 on Gas-Crom Q. This is the same packing that Desmoras et al 1974 recommend for one of their columns (see Table 7), and as a result meant that phosalone could be determined using the same GC model and column as was used for permethrin. Once the optimum configurations were established on the GC for phosalone, a calibration line for standard concentations required to be found. The concentration of unknown phosalone samples could then be determined by reference to the standards.

2.3.2.2 Results

The optimum conditions determined for phosalone chromatography using the Packard model 438 GC are described in Table 8. The attenuation setting of the GC varied according to the volume and concentration of the sample injected. Where necessary, this is specified. Figure 5 is an example of a chromatogram obtained from injection of 6 ul of a 6 mg/l standard. 36ng

Stock solutions were prepared by dissolving 0.01 gram of phosalone in 100 ml of hexane (Rathburn Glass Distilled Grade) and diluting to obtain the desired concentrations. Three sets of experiments were conducted on the calibration of phosalone, each involving preparation of fresh standards and individual analyses.

The first calibration experiment involved determination of six standards ranging from 12.1 to 2.1 mg/l (determined by weight). The GC attenuation was set at 7 for each analysis. Analyses was carried out four times, in order to check the reproducibility of results. A Spectra Physics SP4100 computing integrator was used to

48 - 8ng

record chromatography and integrate area under peaks. Results for calibration injections are contained in Table 9. A calibration graph (a plot of area against concentration) for each batch of results is plotted in figure 6.
The initial results obtained on analysis of 4 ul injections of the standards were very inconsistent and suggested that an upward drift occured over time (from 8.30 a.m when the GC was switched onto full power, until 5.00 p.m when it was put on standby overnight). The calibration plot was found to be non-linear and curved. Following on from this, 6 ul injections were found to give more reproducible results, although an upward drift was still noticeable. Fairly linear calibration lines were obtained from the last two batches of results.

The second calibration experiment involved determination of seven freshly prepared standards (determined by weight), ranging from 10.8 to 0.9 mg/l. This second experiment was divided into two sets of analyses. The results obtained are listed in Table 10 and are plotted in figure 7. The first analysis produced an uneven calibration line, being neither linear nor a regular curve. The second part of the experiment gave fairly reproducible results and produced a well fitting curve. The results in this case showed a downward drift rather than the upward trend found in the first calibration experiment.

The final calibration experiment involved determination of four standards (determined by volume), ranging from 10 to 1 mg/l. This experiment also consisted of two sets of analyses. The results are found in Table 11 and are plotted in figure 8. The first set were analysed at a GC attenuation of 6, whilst the second set were analysed at a setting of 8, after allowing the GC to rech a stable state (ca. 5 hours in this instance). The results obtained for the first determination, gave a fairly linear calibtation plot, although the 5 mg/l standard had rather a high value. The second set, gave results which were quite reproducible and did not show any réal upward or downward drift trend. When plotted, the 5 mg/l standard was again outwith the reasonably well fitting linear line.

60 - 6 mg/l
4 in 6 µl 1 in 10 = 1 mg/l.

2.3.2.3 Discussion

The results obtained from the calibration experiments indicate that standard results may vary a great deal between injections. As a consequence, care must be taken when using them as reference to calculate unknown concentrations of phosalone samples. Standards should be immediately injected before and after a sample injection, with the mean result taken for calculation of the sample concentration. Where possible, standards similar to the sample area should be used. Error is possible due to injection technique, so it is therefore very important that the analyst is comfortable with the syringe used. A method should be developed by the individual for injection of samples which is sustainable for each injection. Alternatively, automatic injection is recommended.

2.3.3 Sediment Preparation

Before beginning work on developing a method for phosalone determination in aquatic sediments it was necessary to obtain unpolluted sediment which could be spiked with phosalone and used as a standard for the experimental analysis

Work in progress on permethrin utilised a very organic (20-21%) sediment obtained from the Botany Pond situated in the grounds of the River Laboratory. A decision had been taken to use this sediment for permethrin because it was known to be uncontaminated with pesticides. Additionally its high organic content meant that any method developed which could yield results with this sediment would work with other aquatic sediments, which generally have organic content of less than 10%. The same argument applies to the measurement of phosalone in sediments.

Preparation of the sediment used for spiked recovery testing : Wet sediment was collected in fine mesh nets and transferred to 2 mm stainless steel sieves. Sieving removed large particles, with the fine particles collected in stainless steel trays, previously washed with distilled water and methanol. The contents of the trays were allowed to settle, and then as much water as possible decanted. The sediment in the steel trays was then dried at 80°C for 24 hours and 60°C for 48 hours. Following drying the sediment was crushed and sieved through 125 ul sieves and stored in glass jars with bakelite tops until required for analysis.

2.3.4 Extraction and Extract Cleanup

2.3.4.1 Introduction

Extraction of a spiked sediment sample produces a very dirty sample which in most cases requires to be cleaned before GC determination is possible. This cleaning process for pesticides usually takes the form of chromatographic column cleanup, often preceeded or followed by a partitioning technique.

Before beginning work on the extraction of phosalone from the spiked botony pond sediment, it was necessary to find a column which phosalone did not irreversibly adhere too and which did not require vast volumes of an appropriate solvent for elution. Once a suitable column and solvent had been found extracted samples required to be tested on the column to ascertain if the cleanup was sufficienly successful to allow GC determination to proceed. Having found a successful method for the extraction and cleanup, various factors (polarity of eluting solvent, amount of activated florisil used, weight of sediment, extraction technique, etc) were altered in an attempt to optimise the recovery.

2.3.4.2 Preparation of column for sample cleanup

The on-going work on phosalone involved a very complicated extract cleanup stage using Bond Elut florisil columns (described by the manufacturers Analtyical International, as disposable solid phase columns for ultraselective chemical isolation). As Desmoras et al 1974 recommended a florisil cleanup for phosalone it was decided to test the Bond Elut columns on phosalone.

Although florisil Bond Elut columns are usually activated in an oven to remove any traces of water which may be present, prior to use, it was decided to test both an activated (>2hours at 100°C) and a non-activated column for phosalone retention. In addition to the column florisil already present, a small amount of activated florisil (> 2 hours at 100°C) was placed above it. The columns were washed with hexane (100 ml) before addition of 1 mg of phosalone in 10 ml of hexane. The columns were then eluted with hexane in successive 20 ml aliquots. The non-activated column produced all the phosalone obtained, in the first elulate, whilst none was found in any of the elulates from the activated column. The activated column was then eluted with 5% acetone in hexane and resulted in all the phosalone being obtained in 120 ml. The yield, however, was five times greater than that obtained from the non-activated column. Based on these results, two fresh columns under the same conditions as before were both eluted with 5% hexane, producing similar recoveries this time. The columns did retain phosalone and so both preparations could be tested for retention of contaminants in extracted samples.

2.3.4.3 Preliminary extraction and cleanup testing

Before extaction could progress a standard method for preparing the spiked sediment had to be established : A sufficient volume of distilled water (80 ml) was added to a 50 gm sediment sample, in a 250 ml round bottom flask (RBF), to produce a slurry, which was shaken on a Stuart scientific flask shaker for 10 minutes at speed 8. A 0.001 mg sample of phosalone in 10 ml of acetone was then added and the contents further shaken for 40 minutes at speed 6. Following this, the flask and its contents were frozen overnight before being freeze dried on a Edwards Modulyo Freeze Dryer.

After freeze drying, the sediment was divided into two 25 gm samples which were extracted with acetone (150 ml each) by shaking (1 hour at speed 7). After allowing the contents to settle, the solvents were decanted and filtered through 2 Whatman GF/C filter papers into a two clean 250 ml RBF's and the volumes obtained recorded (100 ml each, in this instance). These were then evaporated to dryness and concentrated in 10 ml of hexane. These were then combined before addition of equal volumes (10 ml) to the activated and non-activated columns (which contained 1/2 cm of activated florisil and had been washed with 80 ml of hexane). The first elulates collected from the addition of the extracts to the columns were analysed using the GC method described earlier, but

showed no traces of phosalone being present. The elulate collected from a further 100 ml addition of hexane to the non-activated column was again found to contain no phosalone. This indicated that phosalone in the extract was retained by the florisil and was not eluted immediately on addition of hexane as it did in the pre-extraction column testing. Both columns were then eluted with 100 ml of 5% acetone in hexane and the elulates analysed. The elulate from the activated column, on analysis was calculated, by referece to a standard 10 mg/l injection, to contain 0.065 mg of phosalone. The expected theoretical yield was 0.33 mg (based on on only 2/3 rds of the original acetone being recovered from the extraction stage). Thus a recovery of 20% was obtained from the activated column. Another 100 ml elution of the solvent mixture did not yield any more phosalone from the activated column. The elulate from the non-activated column, was, however determined to contain 0.303 mg of phosalone, which was effectively a recovery of 91%. From these results it was clear that the non-activated column cleanup was the best procedure to continue with.

2.3.4.4 Method testing

From the preliminary extraction and cleanup work, a method for extraction of phosalone from aquatic sediments had been found. This method then required to be tested over a range of concentrations and altered, where possible, to optimise the recoveries.

Tables 12-17 contain a summary of the experiments which were subsequently carried out.

The first experiment (experiment 10a and 10b, Table 12) involved a double extraction of a 1 mg spiked sediment sample. The sample was extracted with 150 ml of acetone by shaking for 1 hour. The solvent obtained after filtration was evapoarated to dryness and concentrated in 21 ml of hexane (added in 3 * 7 ml aliquots with subsequent flask shaking for 10 minutes on each addition). This was then added to the pre-washed column, before elution with successive 20 ml aliquots of 5% acetone in hexane. GC analysis of the extracts revealed no phosalone in the elulates collected from the extract addition to the column nor in the following post addition hexane wash. Analysis of the elultes collected from the solvent elution yielded 0.629 mg for the first extraction and 0.335 mg for the second extraction. This gave a total recovery 97% and verified the original method.

In the next experiment (exp.11. table 12) 200 ml of acetone was used for extraction instead of 150 ml. The solvent obtained from filtration was very cruddy and as a result a 60 ml aliquot was taken and evaporated to dryness instead of the total 140 ml. After cleanup and elution, GC analysis of the elulate gave a yield of 0.383 mg. Calculating for the original 200 ml of acetone gives an total yield for the sample of 1.28 mg, a 128% recovery. From this result it was felt that the volume of acetone used for the simple extraction had been excessive, resulting in a very cruddy sample which even after cleanup was still unsuitable for the sensitive electron capture detector. This was thought to account for the high recovery figure obtained.

The third experiment (exp.12. Table 13) involved extraction of a 0.05 mg spiked sediment sample (50 g) under the same conditions as the tested method. A recovery of 0.021 mg was obtained for the 90 ml volume of recovered acetone. Calculating for the 150 ml original acetone, the actual yield was 0.038 mg, which was effectively a 78% yield.

Soxhlet extraction of the same concentration of sample (0.05 mg) was tested in the next experiment (exp.13. Table 13). This involved 100 condenser refluxes with approximately 250 ml of acetone A soxhlet extraction results in no loss of solvent and therefore requires no back calculating as is required for a simple single extraction. The amount of activated florisil was varied in this experiment, with 1 g being used and a small amount of anhydrous

sodium sulphate included also in the column. 0.038 mg of phosalone was obtained, a recovery of 76%. This indicates that the back calculation made to obtain the total yield in the previous experiment was valid.

The fifth experiment (exp.14. Table 13) tested a lower polarity eluting solvent. In this case 1.5 g of activated florisil was used with a small amount of anhydrous sodium sulphate. Elution with 120 ml of 0.5% acetone in hexane resulted in no phosalone being eluted so a further 120 ml of 1% acetone in hexane was used. This resulted in 0.630 mg of phosalone being recovered, which on calculation from the original acetone gave a total recovery of 1.05 mg, a recovery of 105%. Although a high yield was obtained the volume of solvent which was required to elute the phosalone seemed excessive.

The next two experiments (exps 15 and 16. Table 14) involved comparison of the shaken extraction with a soxhlet extraction. The conditions for both methods were constant. Both 10 g sediment samples contained 0.01 mg of phosalone. Based on the previous experiment, 2% acetone in hexane was used as an eluting solvent, with 1 g of activated florisil and 1/2 cm of the anhydrous sodium sulphate contained in a non-actiavated column, used for the cleanup. The phosalone obtained for both samples was recovered by two 20 ml elutions. The yields obtained were, however, very low with 27% and 36% recoveries obtained for the simple extraction and soxhlet extraction methods respectively. Further elution with 5% acetone in hexane did not reveal any more phosalone.

The next experiment (exp.17. Table 14) used the same concentration of sample as the previous experiments , on which a simple extraction with 50 ml of acetone was carried out. The cleanup utilised a smaller amount of activated florisil and contained no sodium sulphate. The total yield of phosalone retrieved was 0.0057 mg, indicating a recovery of 57%. This is twice the recovery obtained from the last experiment, indicating that 5% acetone is a more efficient eluting solvent.

Given the results from the previous experiments it was decided to select the method which seemed to be most successful and carry out double extractions on a range of concentrations (0.1, 0.01 and 0.001 mg), in 10 g sediment samples. 50 ml acetone extraction was followed by filtration and column cleanup in which 1 g of activated florisil was used. 5% acetone in hexane was used for the elution of the phosalone . For the 0.1 and 0.01 mg spiked samples, a recovery of 94% was obtained. A recovery of 57% was found for the 0.001 mg sample.s

2.3.5 Discussion

2.3.5.1 Recommended method

From experimental work, a method for determination of phosalone in aquatic sediments has been established. Using this method, recoveries of greater than 90% have been obtained for phosalone concentrations in Botony Pond sediment, ranging from 20 ug/g to 1 ug/g. The present method will also detect greater than 50% of of the phosalone in a 0.1 ug/g sample.
The recommended method is as follows:

Single Extraction and Filtration

50 g (or 10 g) of sediment and 150 ml (50 ml) of acetone are placed in a 250 ml Round Bottom Flask (RBF) and shaken for 60 minute at speed 8 on a Stuart Scientific Flask Shaker. After allowing the flask contents to settle the solvent layer is decanted and filtered through a Buchner Funnel using two Whatman GF/C filter papers, into a clean 250 ml RBF and the volume obtained recorded.

Extract Cleanup

The solvent obtained from extraction is evaporated to dryness on a rotary evaporator. 3 successive volumes of 7 ml of hexane are added to the dried flask contents and shaken each time for 10 minutes at speed 7, ready for addition to the prepared column.

To a non-activated florisil Bond Elut column 1 g of activated florisil (> 2 hours at 100°C) is added. The column is eluted with 100ml of hexane, before addition of the 21 ml hexane extract. The column is then eluted with 100 ml of hexane before elution with 60 ml of 5% acetone in hexane, the eluate of which is collected in a 250 ml RBF. After collection the eluate, is evaporated to dryness, then taken up in 20 ml of hexane (this is dependant on the concentration of the sample, it may be less according to the sample area which is expected). The sample is now ready for GC determination.

GC Determination

Using the GC configurations detailed in ~~Table 3~~, a 6 ul phosalone standard, commensurate in area with the expected sample area, is injected before and after the 6 ul injection of the sample. Given the mean area for the standards, the concentration of the sample may be deduced, and the sample yield calculated as a result.

2.3.5.2 Recommendations for further work

Further work is required to increase the yields obtained for the less concentrated phosalone samples (0.1 µgm/gm). However, before altering the tested method, it is suggested that a less organic sediment is obtained and used in spiked recovery tests for low levels of phosalone. It is possible that more satisfactory recoveries could be achieved by using the more common sediments, which are generally less than 10% organic.

Not a great deal of the experimental work involved soxhlet extraction and it is suggested that further experiments test this technique. A less polar extraction solvent should be used to decrease the amount of crud which is extracted.

An alternative to soxhlet extraction, which would increase the volume of extraction solvent recovered, would be centrifuging the extraction mixture before decantation and filtration. This should effectively produce a less crudded sample and aid increased recoveries.

During the experimental work performed, it was noticed that the Electron Capture Detector was very sensitive to cruddy samples. As a result it is suggested that the detector is cleaned frequently either by ultrasonicating the nickel foil in hexane, or by increasing the detector temperature sufficiently to burn off the crud which has accumulated from sample injection.

2.3.5.3 Conclusions

A method has been developed which determines phosalone in aquatic sediment. Recoveries for the higher concentrated samples are adequate (greater than 90%), while lower concentrations giving recoveries of over 50% require further investigation. Suggestions for improvements have been made. To the best of my knowledge, the tested method for phosalone determination in aquatic sediments is original.

2.4 Adsorption of phosalone onto kaolinite

2.4.1 Introduction

An experimental study on the adsorption of phosalone onto kaolinite was initiated as part of a wider project, concerned with the sorption of lypophilic compounds onto suspended and sediment particles, and the associated possible risks to benthic invertebrates. By determining the minerals on which phosalone was adsorbed, the susceptible sediments could then be identified and appropriate action taken. While employed at the River Laboratory, under the supervision of Dr W.A. House, I carried out experimental analysis to determine the adsorption of phosalone onto a prepared potassium form of kaolinite. The kaolinite was prepared, using a method based on that described by Hanna and Somasundaran 1979.

Before beginning work on adsorption onto potassium kaolinite, various factors needed clarifying, such as the solubilty of phosalone in water,in buffer solution and the effect of pH.

Using a DU-8 Spectrophotometer the absorbance of phosalone was measured at different concentrations of phosalone. An initial scan of a phosalone solution revealed 3 absorbance peaks at 330.8, 281.7 and 234.2 nm, the latter was found to be the major absorbing wavelength. Difficulty was found in establishing the the solubility of phosalone in distilled water, and solutions of 10 mg/l and 5 mg/l were found to only partially dissolve (determined by absorbance scans). In an attempt to simulate river water conditions, solutions of phosalone in 10 mM potassium dihydrogen carbonate were prepared. Again it was found that phosalone would not fully dissolve at similar concentrations, In the buffer,the phosalone solution had a yellowish appearance which on heating became more pronounced. Experiments were then carried out to try and establish what the yellow appearance signified. 4 solutions contaning 10 mg/l of phosalone in 10 mM buffer, under constant conditions, were heated and stirred for 15,30,45 and 60 minutes respectively on a Moss of Maldon magnetic stirrer at 50°C on speed 6. Scans of each solution were performed frequently over a period of week and subsequently 2 months later. The initial scans revealed that as the duration of heating increased, so the size of the peak absorbances increased correspondingly, with the 60 minute solution (solution 4) producing a very prominent peak at the 330.8 nm wavelength. Two months later all the solutions had also produced this peak. The scans of some of these solutions are shown in figure 9. This 330.8 nm peak is thought to be the substituted phenoxasin found also in the degradation of phosalone in soil (Desmoras et al 1967) and by microorganisms (Golovleva et al 1983). This indicates that heating the solution merely accelerated the hydrolysis process of phosalone in water, which is catalysed by the buffer, potassium dihydrogen carbonate.

The supernatant of a solution, which originally contained 10 mg/l

of phosalone in 10 mM of buffer, was analysed for the effect of pH variation on absorbance. The pH was varied by addition of nitrogen and carbon dioxide to the solution. The absorbance results are recorded in Table 18 and show that phosalone concentration in water is not pH dependant.

After establishing the configurations for phosalone on the GC, further study of phosalone solubilty by hexane extraction was possible. Three solutions were prepared each containing 20 mg/l of phosalone, two of which were in 10 mM buffer and the other in distilled water. All the solutions were stirred without heat for various lengths of time. The solutions prepared with buffer were left stirring for approximately 21 and 148 hours, and after extraction and analysis, were determined to contain 3.35 and 4.31 mg/l of phosalone respectively. The other solution in distilled water, which was stirred for 70 hours was found to contain 4.07 mg/l of phosalone. On no other occasion was any solution of phosalone found to contain more than 4.31 mg/l of phosalone. From these resuls it is concluded that the maximum solubility of phosalone in water or buffer is approximately 4.0-4.3 mg/l.

Having established the solubility of phosalone and its pH independance, work on its adsorption onto the potassium form of kaolinite was started.

2.4.2 Method

Experimental work on the adsorption of phosalone onto the potassium form of kaolinite was carried out using a flow cell designed by Dr W.A. House and C. Kowalczyk. The flow cell, illustrated in figure 10, is operated from the Apple computer using a program specifically written for it, called the Automated Adsorption Control Program (AACP).

The equipment consists of a main cell, into which the adsorbate and buffer are placed, which is connected to the syringe pump and spectrophotometer via a valve. Solution is flowed through the equipment by commands from the microcontroller, WHISPER

The principle behind the operation of the equipment is very simple: It consists of the WHISPER setting the flow sequence to begin. After 3 flows, the WHISPER signals the Apple to take 3 readings of absorbance from the spectrophotometer which are then sent to the Apple and stored on disk. At this point, if required, the autoburette titrates a known volume of solution into the cell and the low sequence is repeated four more times before beginning the overall sequence again.

Work on the adsorption of phosalone on kaolinite required that the background absorbance of the buffer was determined followed by a calibration with a membrane filter in place.

2.4.3 Results

2.4.3.1 Phosalone calibration

Using the flow-cell method, the backgound absorbance of phosalone was determined. This involved obtaining a calibration for the addition of phosalone to the potassium dihydrogen carbonate buffer, which the kaolinite would be suspended in during the kaolinite adsorption experiment. A solution of phosalone in the buffer was prepared and its concentration determined to be 3.35 mg/l by GC analysis. An approximate 200 ml aliquot of freshly prepared 10 mM buffer in a flask was weighed before and after addition to the cell. Before addition of phosalone, the experiment was initiated to ascertain the background absorbance of the buffer and to determine if any drift occurred. The absorbance of the buffer was found to be stable, so addition of the phosalone was begun by programming the autoburette to titrate 2.5 ml in each sequence flow (every 20 minutes). After 27 ml of phosalone had been added the experiment was stopped and the results analysed. Using a program within the AACP, calibration of absorbance to concentration was obtained. Table 19 contains these results while figure 11 shows a plot of the absorbance against concentration.

2.4.3.2 Phosalone calibration with membrane filter

In the kaolinite experiment, a membrane filter was required to prevent any of the sediment escaping from the cell. It was necessary to ascertain if phosalone adsorbed onto the surface of the membrane filter prior to the adsorption experiment with kaolinite. Using a Sartorius 11358 0.1 um membrane filter paper which had been boiled to remove wetting agents, the calibration experiment described above was repeated. The background absorbance of the buffer with the filter in place was found to be stable. From comparison of the calibration results (Table 20) and calibration plot (figure 11) with those obtained without the membrane filter, it was shown that the filter had adsorbed phosalone The amount adsorbed on the filter paper was determined using another program within the AACP and from the results (Table 21), a plot of adsorption against concentration gave the adsorption isotherm of the filter. This is shown in figure 12.

2.4.3.3 Phosalone adsorption onto kaolinite

Before beginning addition of phosalone to the flow-cell, the drift of absorbance of the solution containing the kaolinite was determined. A flask containing an approximate 200 ml aliquot of buffer was weighed before and after addition of the solution to the cell. To the cell 1.2709 gm of the potassium kaolinite was then added and the absorbance drift measured. After a drift was established, the addition of the 3.35 mg/l phosalone solution was

commenced. Using a program within the AACP the adsorption by the filter and kaolinite was determined (Table 22). Comparison with the adsorption on the filter alone, gave the adsorption of phosalone on the kaolinite (Table 23). The adsorption isotherm for the filter and the kaolinite adsorption is plotted in figure 12. Figure 16 shows the adsorption isotherm for the adsorption of phosalone onto kaolinite.

2.4.4 Discussion

From the experimental work, it was found that phosalone adsorbed onto the potassium kaolinite. The relationship between the amount adsorbed (na) by an organic substance and its concentration (c) is often expressed in the form :

$$na = k\ c$$

Where k is the adsorption coefficient (or Henry's law constant). Using the figures obtained for the adsorption isotherm, the adsorption coefficient for phosalone on kaolinite was determined. From the results (Table 24), it was found that the adsorption coefficient was not a constant, as was expected, but decreased with increasing concentration. It is expected that further concentration increase would result in a plateaux being reached. The results stress the need for care to be taken in calculating an adsorption coefficient from an initially linear isotherm at low concentrations and applying the results to predict adsorption at higher concentrations. Further work is needed to clarify the solubility of phosalone in water and its degradation products. Adsorption of phosalone onto the membrane filter is not satisfactory when working at such low concentrations. It is therefore recommended that a filter which does not adsorb phosalone, e.g. a teflon or polycarbonate filter may be suitable, is acquired, before continuing further adsorption experiments.

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4.0 Acknowledgements

I would like to thank all the staff at the River Laboratory for making my placement very pleasant. Special thanks to Dr W.A. House, I. Farr and D. Orr for their kind help and supervision on the phosalone project, which I enjoyed very much. Extra thanks go to I. Farr for the lunchtime table tennis lessons.

APPENDIX

Table 1 Percentage of total area sprayed in the U.K. by each of the groups of the pesticides (Chapman and Long 1985, 1986)

	Organochlorine	Organophosphate	Carbamates	Pyrethroids
1983	0	72	24	3
1984+	0	73	19	8
1985*	0.03	74	20	5
1986&	0.06	69	19	11

\+ raised from 85 per cent of returns
* raised from 95 per cent of returns
& raised from 97 per cent of returns

Table 2 Usage of pesticides on cereals, field crops and peas/beans during 1985 and 1986. Only those compounds which were used to treat areas greater than 500 ha in total are listed. (Chapman and Long 1985, 1986)

Insecticide/ molluscicide	Treated area/ ha		Percentage area of total treated area	
	1985	1986	1985	1986
Phosalone	51009	26727	35.8	34.2
Dimethoate	25352	12329	17.8	15.8
Pirimicarb	21336	13010	15.0	16.6
Demeton-S-methyl	20778	9575	14.6	12.5
Methiocarb	8189	2587	5.7	3.3
Triazophos	6580	4032	4.6	5.2
Permethrin	3478	3064	2.4	3.9
Fenvalerate	2004	1153	1.4	1.5
Oxydemeton-metyl	893	1033	*	1.3
Heptenophos	823	----	*	----
Cypermethrin	800	2018	*	2.6
Metaldehyde	655	524	*	*
Deltamethrin	616	761	*	*
Alphamethrin	----	1246	----	1.6

* less than 1%

Table 3 Solubility of selected pesticides

Pesticide	Solubility, Sw, in water, mg/l	Reference/s
Phosalone	2.15-10	Freed et al, 1979 Desmoras et al, 1974
Pirimicarb	2700	Martin and Worthing, 1983
Dimethoate	25000	Martin and Worthing, 1983
Demeton-S-methyl	3300	Wagner and Thornton, 1977
Permethrin	0.02-0.07	Fujie and markel, 1977

Table 4 Crops and insects to which phosalone is applied to in the United Kingdom upto 1987.
(The UK Pesticide Guide 1988)

Insects	Crops
grain aphid	cereals
seed weavil, brassic pod midge	oilseed rape, brassic seed, fodder rape seed cabbage seed kale seed mustard
pollen beatles	brassica seed mustard
red spider mites	apples pears plums
aphids, codling moth, tortrix moth, winter moth, byrobia mites	apples pears

Table 5 The toxicity of phosalone to various organisms, including the route of uptake.

Organism	Route	LD50(mg/l)	References
mouse	oral	73-205	1,2,3
female rat	oral	135-170	1,2
male rat	oral	120	1
female rat	percutaneous	390-1500	1,2
guinea pig	oral	82-150	1,2
rabbit	percutaneous	1000*	1,2
pierris brassicae (cabbage butterfly)	-----	2.1-2.4£	3
harlequin fish	-----	3.4£	4
mosquito fish	-----	0.2-0.3£	5
daphnids	-----	0.2-0.3£	5

* reference 1 value for LD20
reference 2 value for LD30
£ concentration in ppm

References for Table 5

1 Colinese and Terry 1968
2 Desmoras et al 1974
3 Dubra and Hameed 1983
4 Alabaster 1969
6 Ambrosi et al 1978

Table 6 Extraction of Phosalone Residue (Desmoras et al 1974)

	Plants	Soil	Milk	Meat	Fatty crops and oils (olive, rapeseed)	Citrus crops
Presampling preparation	Slice if necessary	Air dry and sieve	None	Mince	None	Slice if necessary
Sample size (gm)	100	100	50	50	50	50–200
Postsampling preparation	None	None	(As for meat)	Grind with anh. Na_2SO_4 and dry ice	(As for meat)	None
Extraction technique	Blending	Magnetic stirring	Blending	Blending	Blending	Blending
Solvent	Acetone	Acetone	Acetone	Hexane–acetone	Acetone	Pentane and/or ethyl acetate
Rate $\frac{\text{ml solvent}}{\text{gm sample}}$	2–3	2	2	2–2.5	2	2–4
Ingredient added to sample during extraction	None	Anh. Na_2SO_4	Dry ice	Dry ice	Dry ice	None
Number of extractions	2	2	2	2	2	2
Type of extract obtained after solvent evaporation	Contains water	Dry	Fatty	Fatty	Fatty	Oily

Table 7 GLC Configurations

Ref.	GLC Model	Column	Detector	Temperature (°C) Inlet	Oven	Detector
1	Aerograph 680 (pestilizer)	1m * 3mm Chromosorb WHMDS (60–80 mesh) 5% Dow 11	ECD with 3H source 90 V D.C	200	195–210	210
1	Aerograph 680 (pestilizer)	1.2m * 6mm Gas-Chrom Q (80–100 mesh) 5% OV 210	ECD with 63 Ni source pulsed mode	260	245	275
1	Microtek Mt 220	1.5m * 6mm Chromoport XXX 5% SE 30	ECD with 63 Ni source pulsed mode	230	200–210	260
2	4BMPF	1.5m * 3mm Gas-Chrom Q (30–60 mesh) 4% OV-101	FPD	250	200	250
3	Toshniwal RL 04/01A	1.2m * 6mm Supersorb 2% silicon OV-17	ECD with tritium source	---	145	200

References to Table 7

1 Desmoras et al 197
2 Kawamura et al 1978
3 Duhra and Hameed 1983

Table 8	GC Configuration
Column :	1m * 4mm I.D. (glass)
packing :	Chromosorb W-HP (100-120 mesh)
Stationary phase :	2% SP2330
Temperature	
Oven :	230
Injector :	230
Detector :	270
Carrier gas :	200 ml/min Nitrogen.
Total gas flow	

Table 9 Results from 1st calibration experiment, divided into 4 seperate sets of results.

Set 1 (05.05.88)
(GC attenuation = 7)
4 ul injections

Concentration	R.T.	Area
12.1	10.4	540655
12.1	10.41	567161
12.1	10.37	623368
10.0	10.37	662834
8.0	10.39	460577
8.0	10.35	325429
6.0	10.37	221320
4.2	10.34	136411
2.1	10.37	61034
4.2	10.34	142223
6.0	10.33	235730
8.0	10.34	235730
10.0	10.34	382151
12.1	10.27	752753*
12.1	10.28	715474*

Set 2 (06.05.88)
(GC attenuation = 7)
4 ul injections

Concentration	R.T.	Area
12.1	12.4	430345
12.1	12.36	546268
12.1	10.42	492677
12.1	12.37	582817
12.1	12.34	651794
10.0	12.36	556411
2.1	10.38	65262
10.0	10.42	594201
10.0	12.36	690842
10.0	12.37	671969
10.0	12.35	677880
8.0	12.36	508152
8.0	10.43	457541
8.0	12.36	449503
6.0	10.39	309121
6.0	12.35	299623
4.2	10.38	200708
4.2	10.35	188319
2.1	10.35	86813
12.1	10.37	887035*
10.0	12.35	762442*
10.0	12.36	812504*

* indicates a plateaux peak chromatogram
due to GC attenuation being too low

Set 3 (09.05.88)
(GC attenuation = 8)
6 ul injections

concentration	R.T.	Area
12.1	10.6	676273
10.0	10.59	584382
8.0	10.58	511046
6.0	10.59	351698
4.2	10.58	183368
2.1	10.58	78709
2.1	10.59	74898
4.2	10.61	246615
6.0	10.56	418801
8.0	10.58	564162
10.0	10.59	684829
12.1	10.59	806729
12.1	10.59	766791
10.0	10.57	677721

Set 4 (10.04.88)
(GC attenuation = 8)
6 ul injections

concentration	R.T.	Area
2.1	10.67	77477
2.1	10.67	81362
2.1	10.67	83903
4.2	10.69	261411
4.2	10.67	280186
4.2	10.69	279536
6.0	12.58	479972
6.0	12.58	506257
6.0	10.7	500509
8.0	12.58	677231
8.0	12.58	690307
8.0	12.58	695610
10.0	10.6	806779
10.0	12.58	828704
10.0	12.58	821034

Table 10 Results from 2nd calibration experiment, divided into 2 seperate sets of results.

Set 1 (11.05.88 morning)
(GC attenuation = 8)
6 ul injections

Concentration	R.T.	Area
10.9	12.34	747620
8.7	12.35	663171
7.8	12.36	656059
6.9	10.34	590014
3.9	10.37	333637
1.0	10.32	47764
1.0	10.35	48030
3.9	10.38	376807
2.4	10.37	137701

Set 2 (11.05.88 afternoon)
(GC attenuation = 8)
6 ul injections

Concentration	R.T.	Area
10.9	12.36	970786
10.9	12.36	973166
10.9	10.37	918734
8.7	12.35	855544
8.7	10.36	812215
8.7	12.37	872334
7.8	12.34	818211
7.8	10.38	819266
7.8	10.35	810134
6.9	12.34	783092
6.9	10.34	743302
6.9	10.33	745756
3.9	10.33	466188
3.9	10.33	477590
2.4	10.32	255971
1.0	10.27	67176

Table 11 Results from 3rd calibration experiment, divided into 2 seperate sets of results.

Set 1 (12.05.88)
(GC attenuation = 8)
6 ul injections

Concentration	R.T.	Area
1	10.47	30537*
2	10.46	87777*
5	10.49	263015*
10	10.10	357325*

Set 2 (13.05.88)
(GC attenuation = 8)
6 ul injections

Concentration	R.T.	Area
1	10.6	31715
1	10.6	35625
1	10.63	34338
2	10.63	88789
2	10.67	87329
2	10.65	87374
5	10.66	433225
5	10.66	445020
5	10.67	442789
10	10.67	763489
10	10.6	808346
10	10.62	793769

* calculated from attenuation 6 to give comparability with set 2 results.

Table 12 Experimental Method Testing

Experiment number	10a (1st Extraction)	10b (2nd Extraction)	11
Spiking Conditions			
weight of sediment :	50 g	----	50 g
Volume of water :	70	----	70
weight added of phosalone :	1.0 mg	----	1.0 mg
shaker conditions :	40' (6)	----	40' (7)
Extraction and Filtration			
Initial volume of acetone :	150	150	200
shaker conditions :	60' (7)	60' (7)	60' (7)
final volume of acetone :	88	144	140
Bond Elut Florisil Cleanup			
amount of activated florisil :	1/2 cm	1/2 cm	1/2 cm
pre-wash hexane volume :	100	100	100
Extract volume :	3 * 7	3 * 7	3 * 7
Post-wash hexane volume :	100	100	100
Eluting solvent :	5% acetone in hexane	5% acetone in hexane	5% acetone in hexane
Volume used :	88	88	60
Volume of elulate :	88	88	60
GC Determination			
GC attenuation	9	9	9
Standard used :	10 mg/l	10 mg/l	10 mg/l
Standard X :	655464	677121	756957
total sample yield :	0.629 mg	0.335 mg	0.383 mg
Calculated yield :	0.629 mg	0.335 mg	1.28 mg
Recovery :	63%	34%	128%
		97%	

*NB all volumes should be read in ml

Table 13 Experimental Method Testing

Experiment number	12	13	14
Spiking Conditions			
weight of sediment :	50 g	10 g	50 g
Volume of water :	70	15	70
weight added of phosalone :	0.05 mg	0.05 mg	1.0 mg
shaker conditions :	40'(7)	40'(7)	40'(7)
Extraction and Filtration			
Initial volume of acetone :	150	250	150
shaker conditions :	60'(7)	100 soxhlet refluxes	60'(7)
Initial volume of acetone :	90	250	90
Bond Elut Florisil Cleanup			
amount of activated florisil :	1/2-1 cm	1 gm + 1/2 cm anh. NaSO4	1.5 gm + 1/3 cm anh. NaSO4
pre-wash hexane volume :	100	100	100
Extract volume :	3 * 7	3 * 7	3 * 7
Post-wash hexane volume :	80	50	100
Eluting solvent :	5% acetone in hexane	5% acetone in hexane	0.5% and 1% acetone in hexane
Volume used :	40	80	120 and 120
Volume of elulate :	10	80	120
GC Determination			
GC attenuation :	9	9	9
Standard used :	1 mg/l	5 mg/l	1 mg/l
Standard X :	63393	784101	63328
total sample yield :	0.021 mg	0.038 mg	0.630 mg
Calculated yield :	0.039 mg	0.038 mg	0.630 mg
Recovery :	78%	76%	105%

* NB all volumes should be read in ml

Table 14 Experimental Method Testing

Experiment number	15	16	17
Spiking Conditions			
weight of sediment :	10 g	10 g	10 g
Volume of water :	15	15	15
weight added of phosalone :	0.01 mg	0.01 mg	0.01 mg
shaker conditions :	40' (7)	40' (7)	40' (7)
Extraction and Filtration			
Initial volume of acetone :	150	250	50
shaker conditions :	40' (7)	100 soxhlet refluxes	60' (7)
Final volume of acetone :	132	250	35
Bond Elut Florisil Cleanup			
amount of activated florisil :	1 gm + 1/2 cm anh. NaSO4	1 gm + 1/2 cm anh. NaSO4	1/2-1 cm
pre-wash hexane volume :	100	100	100
Extract volume :	3 * 7	3 * 7	3 * 7
Post-wash hexane volume :	100	100	100
Eluting solvent :	2% acetone in hexane	2% acetone in hexane	5% acetone in hexane
Volume used :	40	40	60
Volume of elulate:	40	40	10
GC Determination			
GC attenuation	9	9	9
Standard used :	1 mg/l	5 mg/l	1 mg/l
Standard X :	72127	504121	127049
total sample yield :	0.0024 mg	0.0036 mg	0.004 mg
Calculated yield :	0.0027 mg	0.0036 mg	0.0057 mg
Recovery :	27%	36%	57%

* NB all volumes should be read in ml

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Table 15 Experimental Method Testing

Experiment number	18a (1st Extraction)	18b (2nd Extraction)
Spiking Conditions		
weight of sediment :	10 g	---
Volume of water :	15	---
weight added of phosalone :	0.1 mg	---
shaker conditions :	40' (6)	---
Extraction and Filtration		
Initial volume of acetone :	50	50
shaker conditions :	60' (8)	60' (8)
final volume of acetone :	35	39
Bond Elut Florisil Cleanup		
amount of activated florisil :	1 gm	1 gm
pre-wash hexane volume :	100	100
Extract volume :	3 * 7	3 * 7
Post-wash hexane volume :	100	100
Eluting solvent :	5% acetone in hexane	5% acetone in hexane
Volume used :	40	40
Volume of elulate :	20	10
GC Determination		
GC attenuation	9	9
Standard used :	5 mg/l	5 mg/l
Standard X :	781398	800619
total sample yield :	0.0722 mg	0.0223 mg
Calculated yield :	0.0722 mg	0.0223 mg
Recovery :	72%	22%
		94%

* NB all volumes should be read in ml

Table 16 Experimental Method Testing

Experiment number	19a (1st Extraction)	19b (2nd Extraction)
Spiking Conditions		
weight of sediment :	10 g	---
Volume of water :	15	---
weight added of phosalone :	0.01 mg	---
shaker conditions :	40' (6)	---
Extraction and Filtration		
Initial volume of acetone :	50	50
shaker conditions :	60' (8)	60' (8)
final volume of acetone :	32	40
Bond Elut Florisil Cleanup		
amount of activated florisil :	1 gm	1 gm
pre-wash hexane volume :	100	100
Extract volume :	3 * 7	3 * 7
Post-wash hexane volume :	100	100
Eluting solvent :	5% acetone in hexane	5% acetone in hexane
Volume used :	20	20
Volume of elulate :	10	5
GC Determination		
GC attenuation	9	9
Standard used :	5 mg/l	5 mg/l
Standard X :	736473	761926
total sample yield :	0.0065 mg	0.0017 mg
Calculated yield :	0.0065 mg	0.0029 mg
Recovery :	65%	29%
		94%

* NB all volumes should be read in ml

Table 17 Experimental Method Testing

Experiment number	20a (1st Extraction)	21b (2nd Extraction)
Spiking Conditions		
weight of sediment :	10 g	---
Volume of water :	15	---
weight added of phosalone :	0.001 mg	---
shaker conditions :	40' (6)	---
Extraction and Filtration		
Initial volume of acetone :	50	50
shaker conditions :	60' (8)	60' (8)
final volume of acetone :	27	45
Bond Elut Florisil Cleanup		
amount of activated florisil :	1 gm	1 gm
pre-wash hexane volume :	100	100
Extract volume :	3 * 7	3 * 7
Post-wash hexane volume :	100	100
Eluting solvent :	5% acetone in hexane	5% acetone in hexane
Volume used :	20	20
Volume of elulate :	5	5
GC Determination		
GC attenuation	8	8
Standard used :	1 mg/l	1 mg/l
Standard X :	726473	766226
total sample yield :	0.00022 mg	0.00018 mg
Calculated yield :	0.00022 mg	0.00297 mg
Recovery :	22%	30%
		52%

* NB all volumes should be read in ml

Table 18 Absobance of Phosalone with varying pH

pH	Absorbance results		
	330.8 nm	281.7 nm	234.2 nm
8.92	0.0575	0.0854	0.1844
8.92	0.0566	0.0856	0.1844
8.56	0.0546	0.0825	0.1858
8.56	0.0542	0.0814	0.1861
8.11	0.0551	0.0818	0.1851
8.11	0.0548	0.0820	0.1863
7.72	0.0551	0.0825	0.1870
7.72	0.0550	0.0825	0.1866
7.77	0.0542	0.0815	0.1865
7.77	0.0541	0.0815	0.1862
7.24	0.0539	0.0818	0.1868
7.24	0.0540	0.0815	0.1896
7.60	0.0560	0.0845	0.1932
7.60	0.0552	0.0836	0.1917
6.97	0.0540	0.0825	0.1896
6.97	0.0545	0.0829	0.1902
6.83	0.0572	0.0854	0.1890
6.79	0.0571	0.0850	0.1886
5.70	0.0559	0.0855	0.1874

Table 19 Phosalone calibration

Concentration (mg/l)	Absorbance	Volume (ml)
0.04175	9E-04	2.5
0.08246	1.8E-03	5.0
0.12235	2.63E-03	7.51
0.16112	3.3E-03	10.01
0.19912	4.0E-03	12.52
0.23608	4.87E-03	15.02
0.27232	5.36E-03	17.53
0.30760	5.93E-03	20.03
0.34207	6.6E-03	22.53
0.37590	7.23E-03	25.04
0.37816	7.03E-03	25.21*
0.41106	7.5E-03	27.71

* Autoburette refilling

Table 20 Phosalone calibration with membrane filter

Concentration (mg/l)	Absorbance	Volume (ml)
0.08598	1.22E-03	5
0.16767	2.33E-03	10
0.24536	3.1E-03	15
0.31949	4.5E-03	20.01
0.39003	5.13E-03	25.01
0.39279	4.93E-03	25.21*
0.45999	5.56E-03	30.21

* Autoburette refilling

Table 21 Filter Adsorption

Concentration (mg/l)	Adsorption (mg)
0.05496	6.04E-03
0.11303	0.0192
0.15476	0.0185
0.23097	0.0186
0.26544	0.0267
0.28903	0.0376
0.33076	0.0435
0.37431	0.0486
0.40879	0.0554
0.45052	0.0554

Table 22 Filter and Kaolinite Adsorption

Concentration (mg/l)	Adsorption (mg/g)
0.03124	9.73E-03
0.06039	0.0194
0.09522	0.0283
0.13939	0.0357
0.18188	0.0430
0.20972	0.0399
0.26589	0.0450
0.30584	0.0517
0.36223	0.0559

Table 23 Kaolinite Adsorption

Concentration (mg/l)	Adsorption (mg)
0.0312	0.0105
0.0604	0.0169
0.0952	0.0218
0.1394	0.0245
0.1819	0.0270
0.2097	0.0208
0.2659	0.0197
0.3058	0.0220
0.3622	0.0199

Table 24 Variation in the Adsorption Coefficient with Concentration

Concentration (mg/l)	Adsorption Coefficient (l/g)
0.0312	0.3365
0.0604	0.2798
0.0952	0.2290
0.1394	0.1758
0.1819	0.1484
0.2097	0.0992
0.2659	0.0741
0.3058	0.0719
0.3622	0.0549

Figure 1:- FRESHWATER BIOLOGICAL ASSOCIATION

RIVER LABORATORY

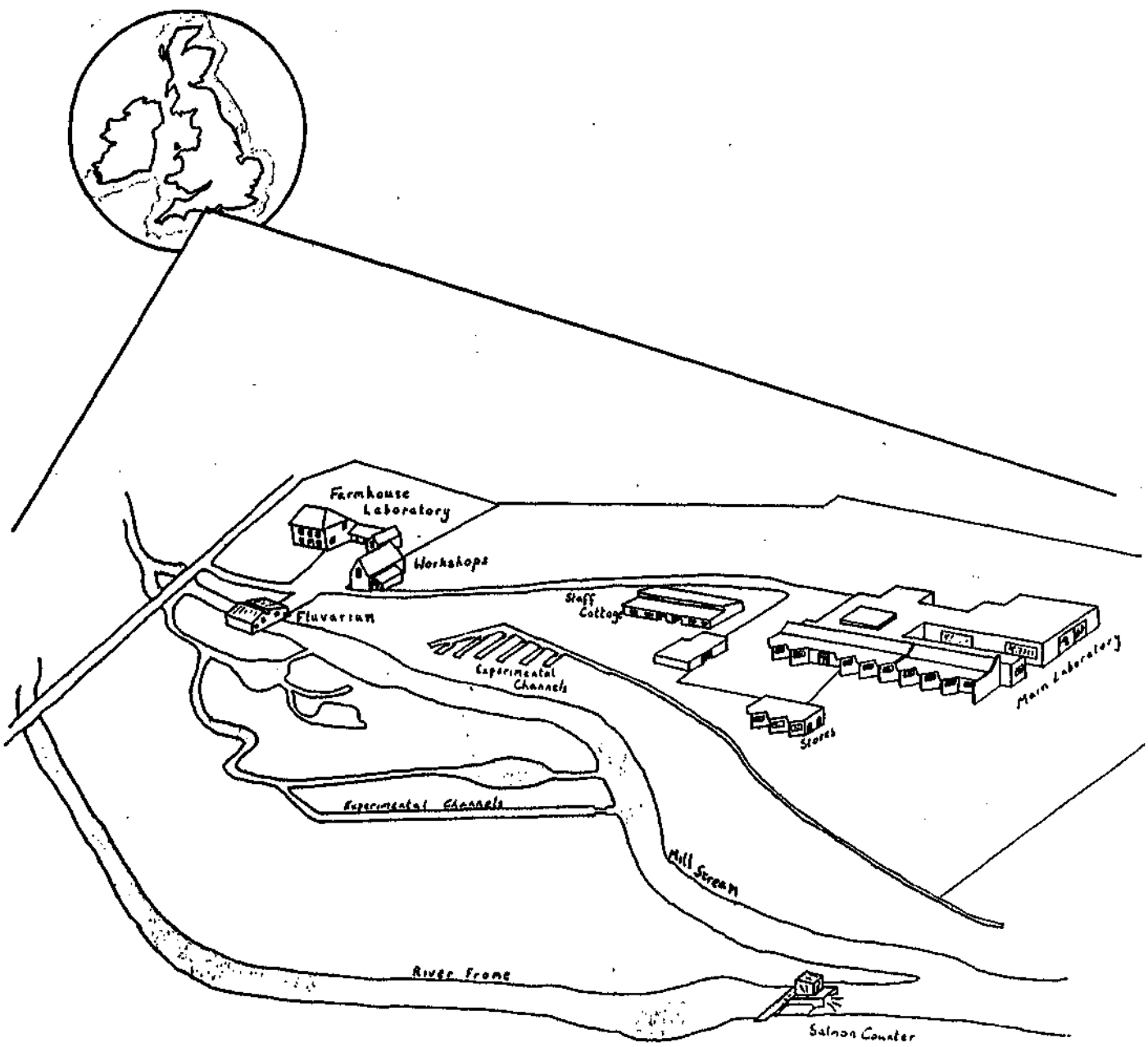


Figure 2 Metabolism of phosalone in plants (Desmoras et al 1967)

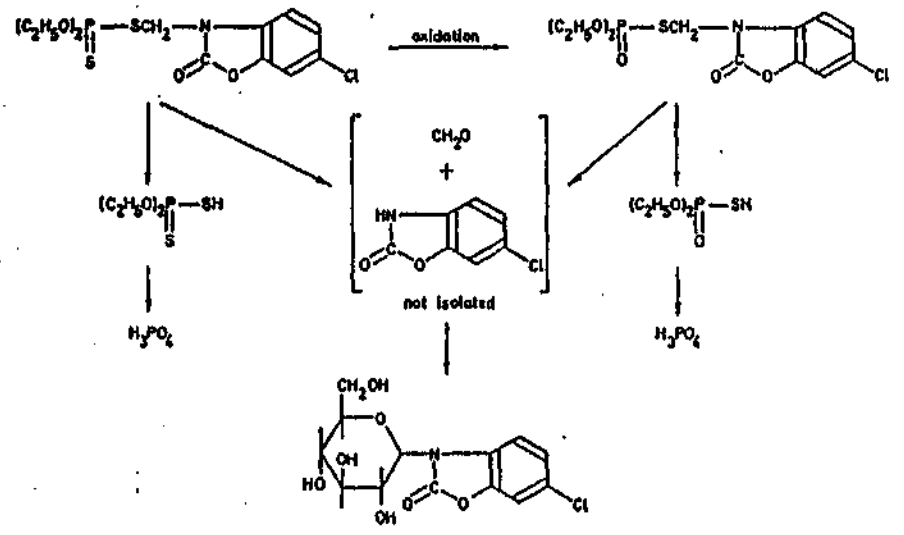


Figure 3 Metabolism of phosalone in soil (Desmoras et al 1967)

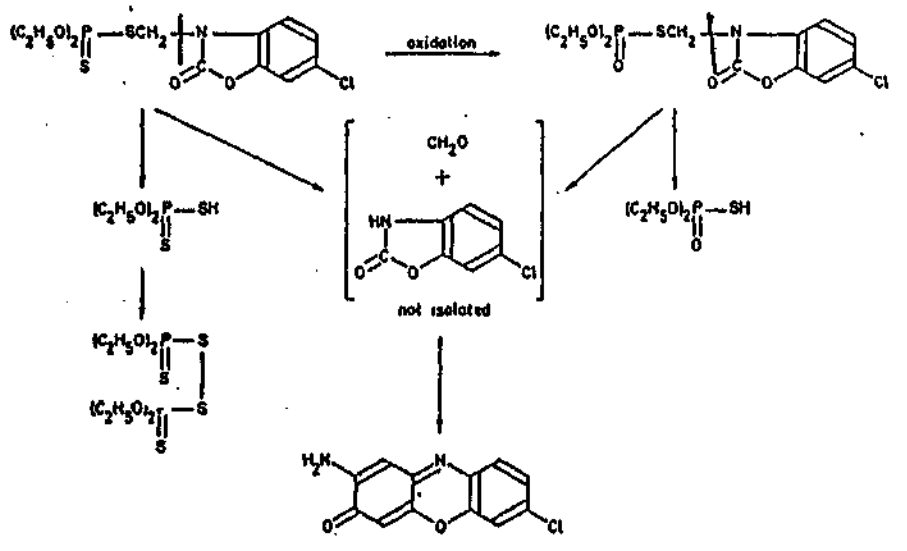


Figure 4 Microbial degradation of phosalone (Golovleva et al 1983)

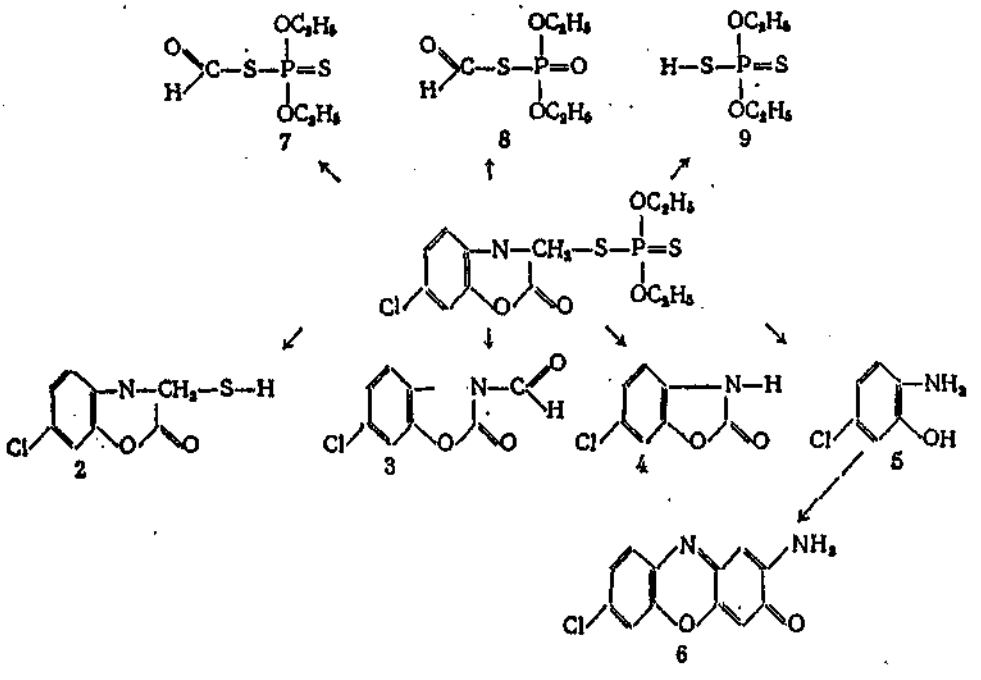
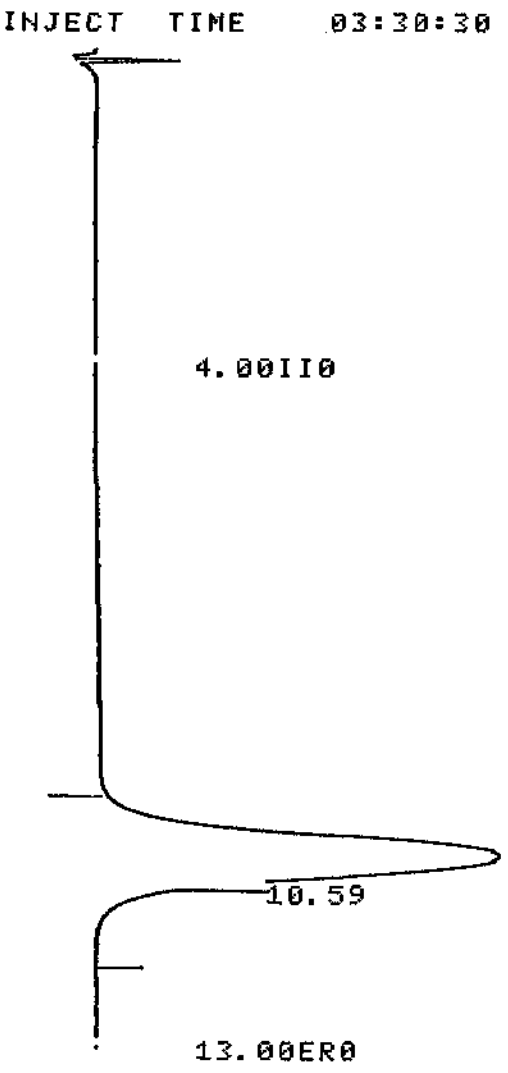


Figure 5 Chromatogram from a 6 ul injection of a 6 mg/l phosalone in hexane standard. (GC attenuation = 7)



PHOSALONE PHOSALON 03:30:30

FILE 1 METHOD 5. RUN 13 INDEX 12

ANALYST: D.MAIN

NAME	CONC	RT	AREA	BC	RF
PHOS	14.212	10.59	351698	01	24747.3
TOTALS	14.212		351698		

Figure 7 Second Calibration Experiment :- Plots of Area Against Concentration

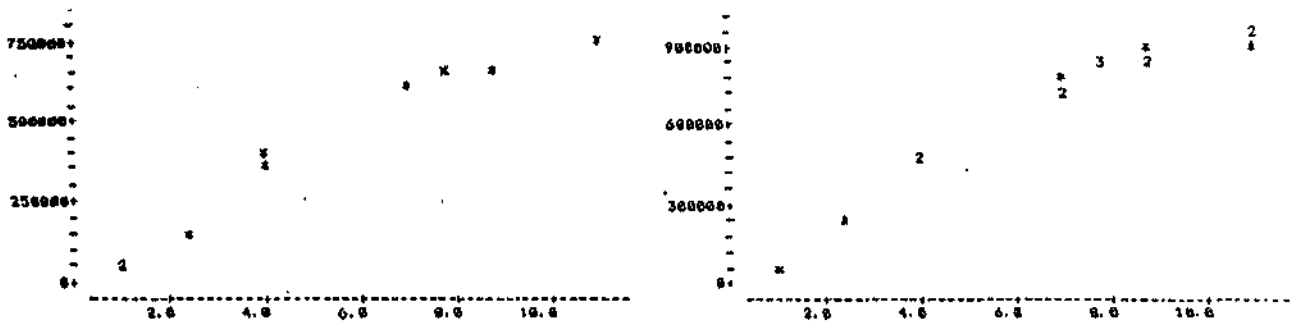


Figure 8 Third Calibration Experiment :- Plots of Area Against Concentration

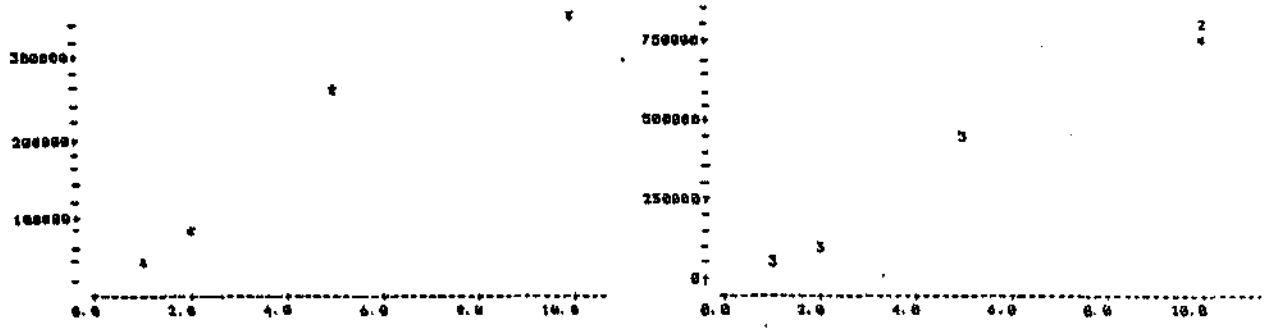
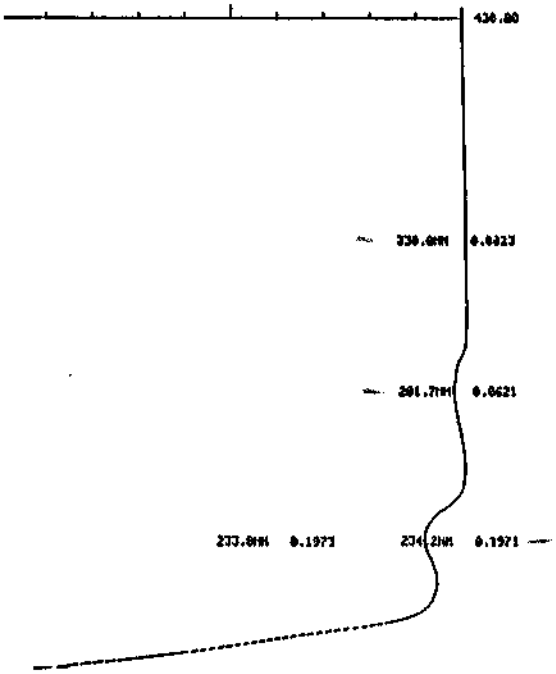


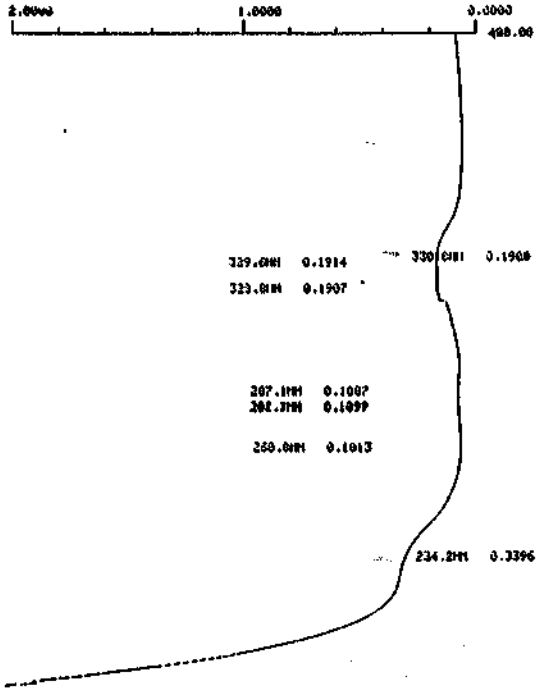
Figure 9 Absorbance scans

04.05.88

solution 1
(15 minutes heating)



solution 4
(60 minutes heating)



07.07.88

solution 1

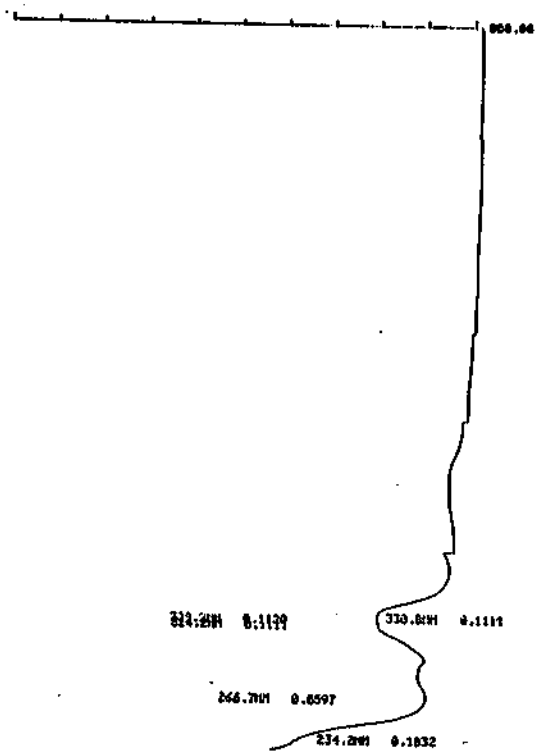
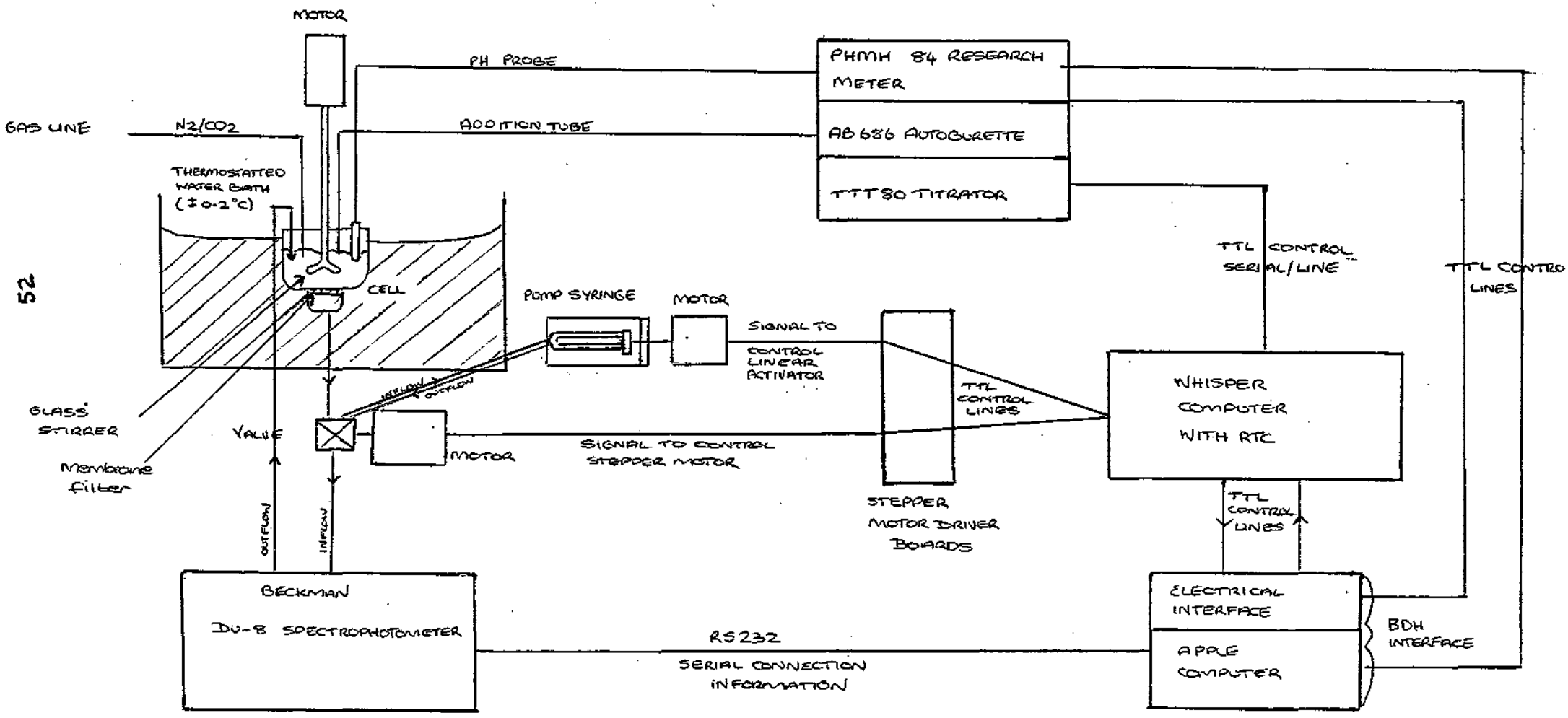


Figure 10 Diagram of Flow Cell



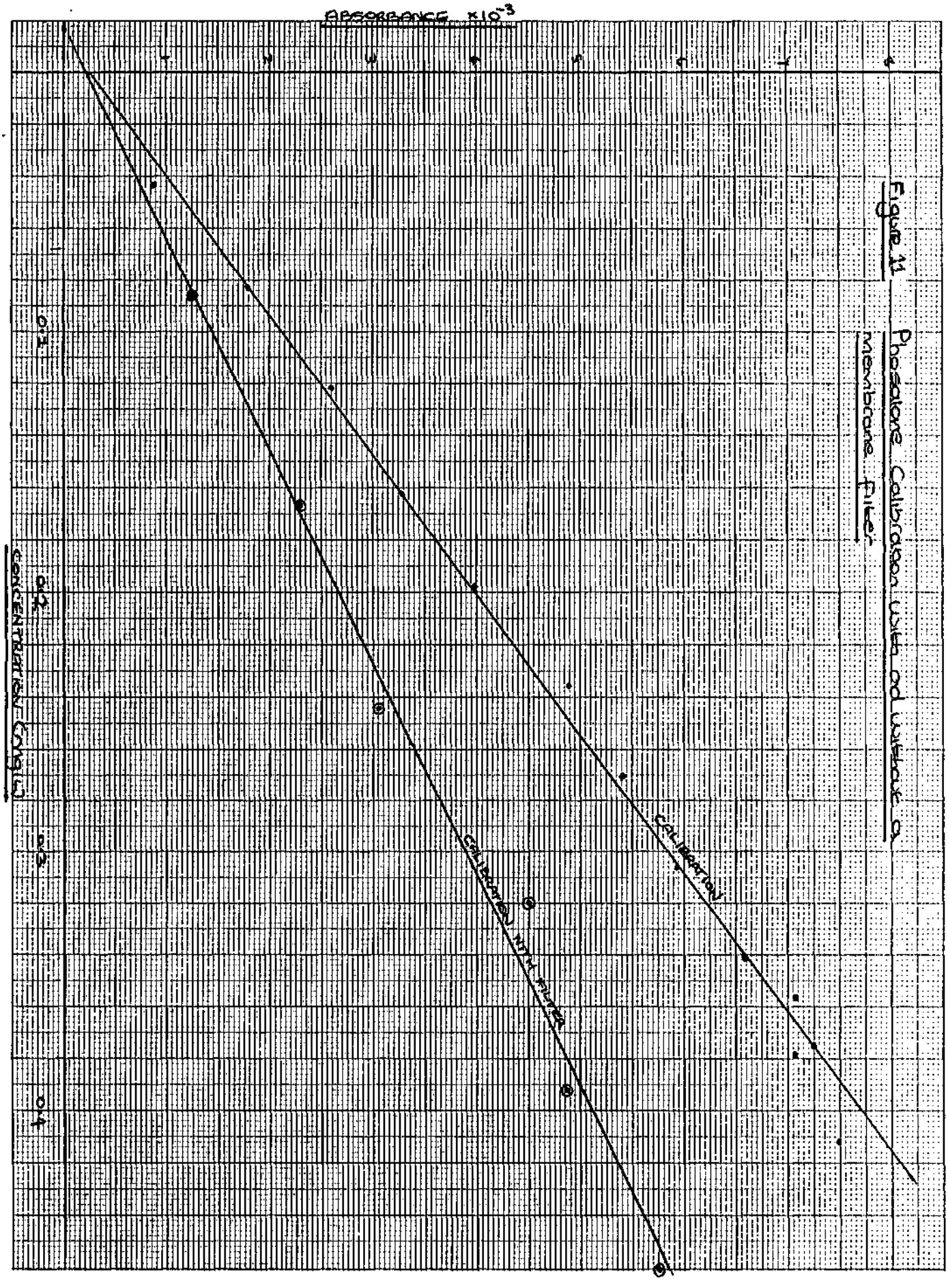


Figure 11 Phosphate Calibration with and without a Membrane Filter

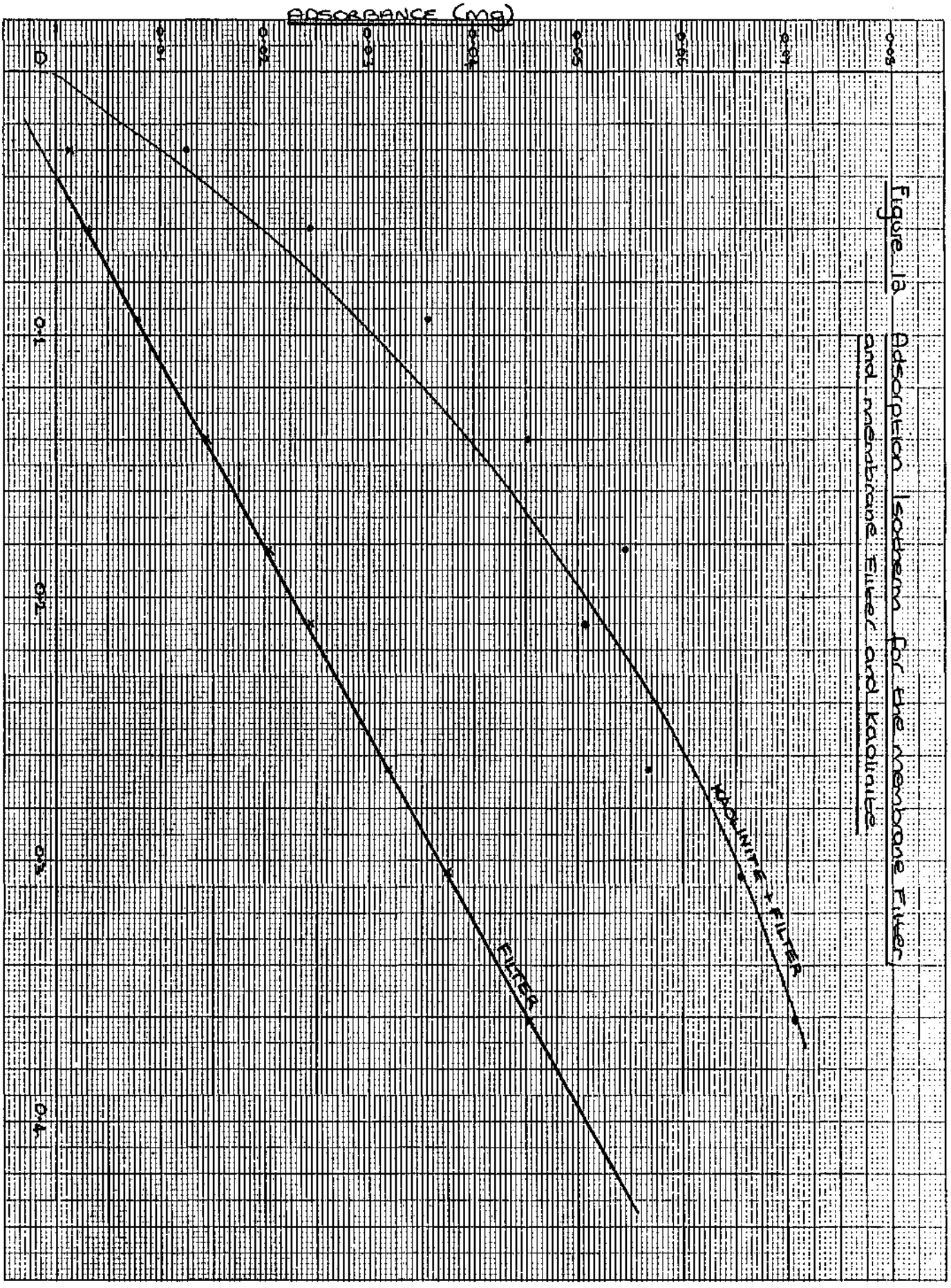


Figure 1a Adsorption Isotherm for the membrane Filter and membrane Filter and kaolinite

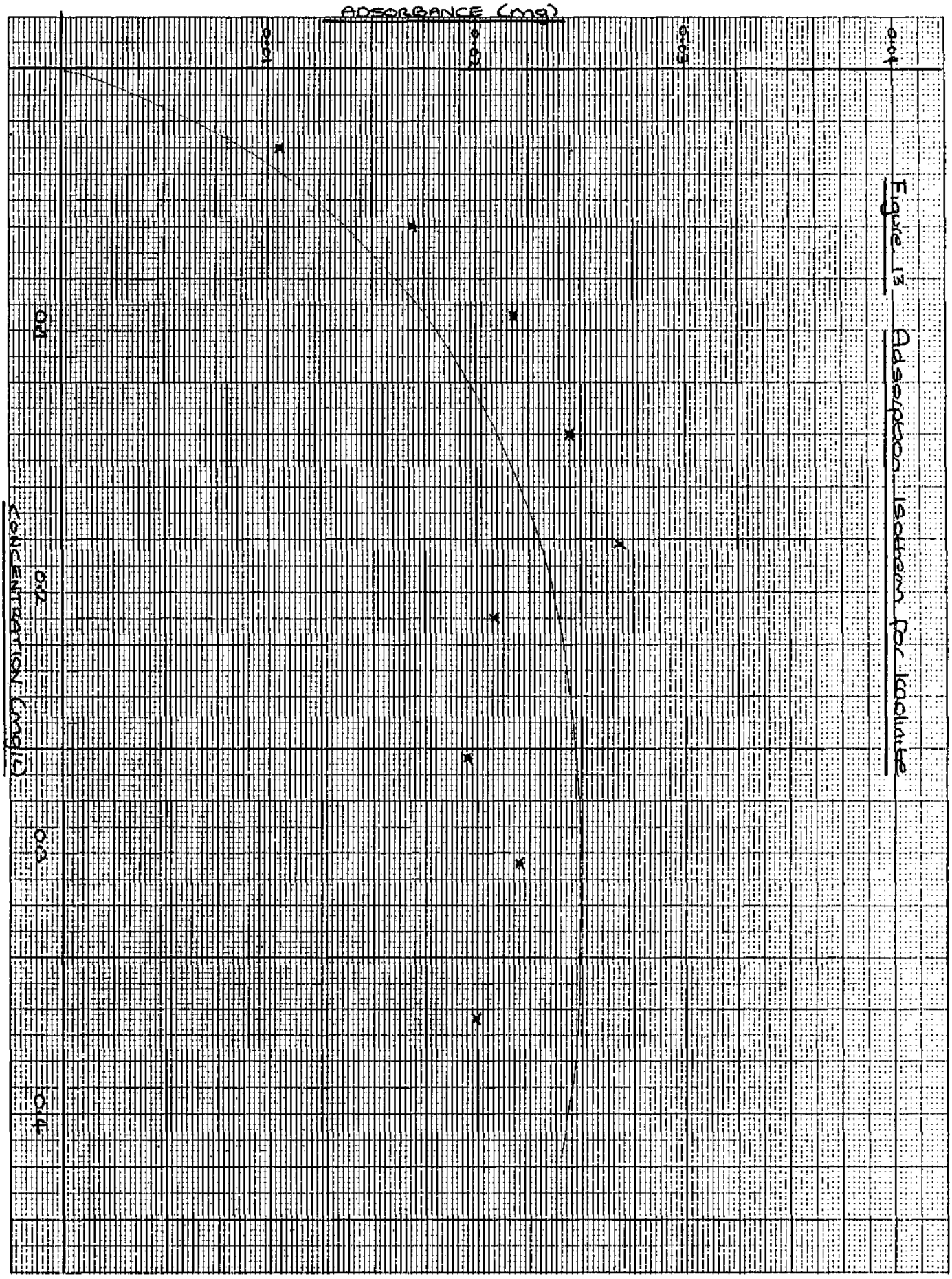


Figure 13 Adsorption Isotherm for Icaltube