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Research and development on procedures to stabilize acaricides in livestock dips

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FOREWORD

Ticks and mites are serious ectoparasites of livestock in many countries. As vectors of animal diseases they pose a threat to livestock production. Traditionally, different types of acaricides are used to control them. One of the most commonly used tick control techniques is to force animals to walk through an acaricide suspension in a trough or Cattle Dip. Dipping is quite effective as the entire body of the animal gets treated with the acaricide. However, with increased usage the concentration of the acaricide in the 'dip' declines due to removal by the animal and degradation by biological and chemicals processes. The dissipation of the acaricide results in loss of efficacy of the 'dip', and may also enhance the development of resistance by the ectoparasites to the acaricides. Maintenance of an effective concentration, by periodic recharge or stabilization of the acaricide, is essential to assure efficient and cost-effective control and to minimize chances of resistance to develop.

In 1990, the FAO/IAEA Joint Division, recognizing the need for co-ordinated research on studying the dissipation of acaricides in cattle dips and developing procedures to stabilize them, established a 5-year Co-ordinated Research Programme on Development of Procedures to Stabilize Acaricides in Livestock Dips and of Simplified Methods to Measure Their Concentration, Using Nuclear Techniques. In initiating this programme, the Joint Division recognized that major gaps exist in the knowledge in this area which, if filled, would greatly aid developing countries in their effort to more effectively use acaricides to protect animal health. This TECDOC reports the accomplishments of this programme.

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SUMMARY OF THE CO-ORDINATED RESEARCH PROGRAMME

Introduction

As vectors of animal diseases, ticks pose an increasing threat to livestock production in many countries [1]. No other group of arthropods transmit such a variety of infective organisms to animals and man, ranging from viruses through bacteria and protozoa to helminths, many of which are the cause of important diseases. The main tick-borne diseases of cattle include anaplasmosis in almost all tropical and subtropical regions, bebesiosis in temperate as well as tropical and subtropical regions, cowdriosis in sub-Saharan Africa and theileriosis in Africa, the Middle East and Asia [2]. FAO has estimated total annual world losses due to tick-borne diseases at roughly US $7000 million, but the total losses due to ticks may be in fact much higher [3]. The use of acaricides for tick control is considered an essential part of an integrated approach, which also includes host resistance, cattle movement, habitat manipulation, and vaccination [4]. The integrated control methods that are successful against single-host ticks in countries like Australia, are confounded by multi-host ticks in Africa. This necessitates the use of acaricides for control of ticks [5]. Acaricides may be applied to the animals as sprays, dusts, pour on, in ear tags and neck bands or in suspensions in vats through which animals are made to pass (dips). The use of dips is the most common method in many countries [6,7,8].

Problem

During use acaricides in dips dissipate. For efficient tick control it is important to maintain the concentration of the acaricide at an effective level, notably to ensure the immediate effect of the treatment but also to reduce the chance of development of resistance in the ticks. Another problem associated with the use of the dips is the disposal of the contents of the used dips to avoid hazards for the environment and non-target organisms.

Background

Acaricides used for tick control on cattle belong to several classes of organic compounds. These include several organophosphates and pyrethroids, but the more persistant organochlorines are also used, although their use is banned in many countries. However, since 1968 the most widely used acaricide in dipping operations in several countries is the organophate coumaphos [9]. In the USA it is used for the control of the southern cattle tick (*Boophilus microplus*) and the cattle tick (*Boophilus annulatus*) by the Animal and Plant Health Inspection Service (APHIS) in its Tick Eradication Programme. Several hundred thousand cattle are dipped annually in approximately 60 dipping vats along U.S.-Mexican border [10]. Each vat contains approximately 15000 L of coumaphos suspension. Vats are emptied, cleaned, and recharged every 6 to 12 months, generating approximately 10 million L of coumaphos waste per year. Research is being conducted on development of waste disposal methods that minimise environmental hazard [10]. The other compounds used for the control of cattle ticks include amitraz, chlorfenvinphos, chlorpyriphos, deltamethrin, cypermethrin and the organochlorine lindane.

Acaricides may dissipate by biological degradation [11] or by physical and chemical processes such as evaporation, hydrolysis, oxidation, photodecomposition, isomerization and adsorption to soil and sediment. The mechanism and rate of dissipation depends on the ambient temperature, pH and air velocity; the type of soil and its content of organic matter; the type of microorganisms and the physical-chemical properties of the acaricide.

An effective concentration of the acaricide in animal dips can be maintained by reducing their rate of dissipation and by periodic recharge. The stability of many acaricides varies with the pH of the dip contents. For example coumaphos is stable at acidic pH. At alkaline pH the pyrone ring of the molecule opens, closing again at acidic pH. Therefore, periodic adjustment to keep the pH below 7 should stabilize the acaricide. Superphosphate fertilizer solution can be used as a buffer to maintain an acidic pH. It is readily available in farms and ranches and, in the absence of other factors, it offers an inexpensive means to maintain coumaphos concentration in the dip at an effective level. Other additives to the dips may also act as buffers and prolong the activity of the acaricides.

The stability of the acaricide suspension depends on the type of formulation. In some formulations the acaricide settles to the bottom of the dipping vat and lower concentrations remain in the dip fluid. However, stirring of the dip content prior to dipping of the animals resuspends the acaricide. Passage of cattle through the dip vats also removes some of the acaricide.

Pesticide analysis generally involves the use of sophisticated analytical instruments which can not be used in the field. However, in dipping vats the concentrations of acaricides are high enough for less sensitive but more robust methods such as spectrophotometric methods and thin layer chromatography. Development of simplified methods would help in monitoring of the acaricide concentration in dips.

Objectives

The primary objective of the programme was to study the dissipation of some of the commonly used acaricides in model dipping vats, to test the effect of buffering agents on their stability and to develop simplified methods for monitoring the concentration of the acaricides in vats. The secondary objective was to generate additional information which would help in the development of procedures for safe disposal of the dip waste. The used dips containing appreciable quantities of acaricides, if not disposed of properly, can pose considerable hazard to human health and the environment. The acaricides and degradation products may contaminate surface water as run off or leach in to the soil and contaminate ground water. Therefore, attention should be paid to decontamination of the used dips and procedures need to be developed to accelerate degradation of residual acaricides in used dips.The knowledge of how to prolong acaricide persistance could also be applied to reduce its concentration prior to disposal of the used dip contents.

Approach

The common features of the programme were to use of model dips with and without recharge and study of the effect of buffering agents, especially superphosphate fertilizer, and sediment on the stability of the acaricides. Model dips were necessary so that ^{14}C-labelled compounds could be used. They were placed out-doors and protected from sunlight and rain, or in-doors under controlled conditions of temperature, humidity and air flow simulating out-doors conditions. Some studies with unlabelled compounds were performed in real cattle dipping vats. The research collaborators chose the acaricides most commonly used in their countries and were free to add other variables to their studies. These included the environmental fate, degradation and mobility in soil columns, dissipation in soil or pond water in biometer flasks, stability under different conditions of pH and temperature and binding to soil.

The Programme

The decrease in the acaricide concentration is normally attributed to removal by the passage of cattle, adsorption on sediments which subsequently settle to the bottom of the dipping vats, volatility and degradation due to microbial activity, chemical and photodegradation. Therefore, there is a need to monitor the dissipation, degradation and binding to the sediment of the acaricide in vats so that no loss of efficacy is experienced.

In a typical study water in a model dip was treated with ^{14}C-labelled acaricide and initial concentration recorded. Two weeks later soil was added to the suspension, to simulate passage of cattle, and samples were taken and analyzed. This procedure was repeated every two weeks for 44 weeks. The sample was filtered or centrifuged and the concentration in the filtrate or the supernatant was determined. Similarly, the concentration of ^{14}C in the sediment was determined by combustion of the soil in a biological oxidizer. At the end of the study residues in the sediment were extracted with solvents and analysed for the presence of the parent compound as well as the metabolites. The stability of coumaphos was studied in most detail as it is the most commonly used acaricide. The dissipation of coumaphos (Panama) and amitraz (Brazil) in commercial cattle dipping vats was also studied.

Three rapid methods were validated for monitoring the concentration of acaricides in field vats.

Results

Test of the stability of coumaphos suspension in a laboratory model dip in Canada showed that 88% of the radioactivity initially applied to the dip was still present at the end of 44 weeks, most of it in the sediment. It included 76% coumaphos, 3.0% chlorferone, 2.7% potasan and 0.7% oxygen analogue of coumaphos. The pH of the suspension decreased from 7 to 4.2 after 44 weeks. On the other hand, in model cement, concrete or plastic vats placed outdoors, the pH of the suspension increased from 7 to 8.1 in plastic (Philippines) vats and to 8.5 in cement (India) or concrete vats (Panama). The concentration of coumaphos in the vat suspension decreased steadily to 21% of the initial concentration after 126 d (Philippines), 15% after 180 days (Panama), and 4.3% after 255 d (India). However, the addition of superphosphate fertilizer at a concentration of 10 g/L at regular intervals stabilized the pH at 5 and reduced the degradation of coumaphos. The time to <50% loss (DT_{50}) of coumaphos in the first phase of dissipation in cement vats was 58-99 d, but extended to 114-172 d on the addition of superphosphate fertilizer. The addition of sodium citrate or copper sulfate did not have any affect on its stability. Chlorferon and potasan were identified as degradation products. In one case a third (India) degradation product, 4-methylumbellifrone, was also reported.

Coumaphos is reported to undergo photolytic degradation with DT_{50} of 191 d at pH7 at 22 °C [12]. A laboratory study showed that exposure to sunlight or U.V. radiation resulted in the precipitation of coumaphos and degradation products. Spent dip solutions are sometimes discarded by spreading on the soil surface. In order to assess the potential of coumaphos to contaminate ground water when disposed in this way its mobility was studied in soil column experiments. The results showed that it remained in the top 10 cm of the soil and did not leach further down. Thus, the acaricide does not have a high potential to contaminate ground water.

Dissipation of lindane in model concrete vats was studied in Bangladesh and India. In Bangladesh it dissipated very rapidly, and after 3 weeks only 3.5% of the original amount remained in the suspension and 9.6% in the supernatant. In India its DT_{50} was 101 d. Degradation resulted in the formation of two metabolites which cochromatographed with 1,2,4-trichlorobenzene and 1,3,5-trichlorobenzene. The addition of cowdung and soil to the vats did not extend the stability of lindane. Similarly, monthly recharging of the dip did not maintain the concentration of the acaricide, which kept decreasing after every recharge. The dissipation was not affected by a change in the pH.

Chlorfenvinphos rapidly dissipated in the presence or absence of superphosphate or copper sulfate. In Ecuador and Zambia the addition of superphosphate reduced the dissipation somewhat but neither this nor recharging was efficient in maintaining a concentration of the acaricide which would be effective in tick control. In Ecuador the DT_{50} of the acaricide in the vats was 6.2 weeks without recharge and 8 weeks with recharge. Addition of superphosphate to the vat extended the DT_{50} from 6.2 to 8.7 weeks. Degradation of chlorfenvinphos in anaerobic soil was studied by using biometer flasks. It showed that chlorfenvinphos degraded with the formation of 2,4-dichloroacetophenone and 2,4-dichlorobenzaldehyde. The acaricide strongly bound to the soil and leaching in the soil columns was very little. In Zambia only 3% of the applied amount of the acaricide leached through the column. Exposure of chlorfenvinphos to sunlight influenced its isomerization from isomer Z to E.

Deltamethrin was stable for 28 d in buffer solution at pH 4,7 and 8 and at temperatures of 14, 28 and 35°C in Brazil but degraded at pH 9, yielding 3-phenoxybenzoic acid and 3-phenoxybenzyl alcohol. After 75 d in a model dip, 60% of deltamethrin was found in the sediment.

After 20 d in the model dips in Brazil 73.8% of amitraz remained undegraded and four months after its application to the top of soil columns, 90% remained, all of it in the top 10 cm. The DT_{50} of chlorpyriphos in the vats in Bangladesh was 22 d.

USDA scientists have identified microbial consortia present in dip wastes and these can be induced to mineralize coumaphos. They are able to colonize plastic fibers in biofilters and can be used in filters to metabolize coumaphos. A field scale biofilter, capable of treating 15,000 L batches of waste dip has been shown to reduce the coumaphos concentration from 2000 mg/L to 10 mg/L in 2 weeks.

Three methods for monitoring the concentration of organophosphorus acaricides in dips were validated and found useful. One is based on the reaction of chlorfenvinphos with 4-(4-nitrobenzyl) pyridine and subsequent spectrometric measurement of the intensity of the colour formed with tetraethylene pentamine. The second method involves colorimetric detection on a filter paper of coumaphos on reaction with a mixture of liver enzyme extract (as source of cholinesterase), potassium hexacyanoferrate and bromoindoxyl acetate. The third method indicates the presence of coumaphos by development of blue colour on reaction of the acaricide with a pyridine containing reagent followed by an alkaline solution [13].

Conclusions

1. The addition of buffering agents such as superphosphate fertilizer reduces the dissipation of coumaphos in dipping vats.

2. Adsorption to soil, sediment and dipped animals is a major factor for the loss of coumaphos from the dip suspension. Agitation of the dip content resuspends the acaricide and restores its effect. Other factors which also contribute to the loss of coumaphos include photolysis and chemical and microbial metabolism, which result in the formation of potasan, chloferone and other metabolites.

3. Amitraz, chlorfenvinphos and coumaphos do not normally leach below the top 10 cm of the soil and, therefore, do not have a high potential to contaminate the ground water.

4. Lindane and chlorfenvinphos in model dips dissipate rapidly and a change in pH is not helpful in their stabilization at an effective concentration. Recharge of lindane is lso not useful in improving its effectiveness information may influence decisions on the continued use of these acaricides in animal dips.

5. The stability of deltamethrin is affected by the pH of the dip and the presence of sediment. Its stability decreases with an increase in the pH and most of it partitions into the sediment.

6. The use of biofilters colonized by microbial consortia present in the dip waste offers the potential to degrade coumaphos from the dip waste before the disposal of the waste.

Outputs

1. Information among scientists from developing countries, Canada and USA was exchanged through participation in research coordination meetings. The scientists from the developing countries received the latest information on the use of acaricides in North America and the technologies with potential for safe disposal of the dip waste.

2. Experience was gained in the design and conduct of laboratory and field experiments, including the design and construction of replicated model dips, preparation of suspensions, addition of sediment to simulate the passage of animals through the dips, addition of superphosphate fertilizer, copper sulphate and other buffering and bacteriostatic agents, monitoring of pH and temperature of the suspension, sampling and analysis.

3. Additional skills were developed in the use of radiotracers, GC, HPLC and TLC for the analysis of acaricides and degradation products and experience was gained in the selection of GC and HPLC columns, gas and solvent flow rates, element specific GC detectors and other parameters for optimum operation of the analytical instruments.

4. Three simple colorimetric methods for monitoring residues of organophosphorus acaricides in field dips was validated.

5. Several participants plan to continue research in this area after the completion of the CRP.

6. The data generated in this programme may be used by local authorities for pesticide registration purposes.

Recommendations

1. The information generated in the programme should be disseminated to the Member States for use by the competent authorities.

2. Any future work on this subject should consider including the following studies:

   - Screening of synthetic pyrethroids and other newer insecticides for acaricidal activity.
   - Validation of the effectiveness of superphosphate fertilizer and emulsifying agents on the stability of coumaphos used in field dips under local conditions.
   - Evaluation of the effect of sprays and pour-on methods on the development of resistance by ticks and study of tick resistance to acaricides by using bioassays.
   - Development of simple and more robust methods, such as those based on ELISA, colorimetric assays, colour strips and cholinesterase inhibition, for monitoring the concentration of acaricides in dips under field conditions.
   - Continuation of investigation on the use of microbial degradation as a means of bioremediation of dip waste until an effective method has been found.
   - Monitoring of residues of acaricides in the milk and tissue of the treated animals.

   Too high residue levels have already led to cases of rejection in crossborder traffic of slaughter cattle in some countries. It is likely to affect the livestock industry in meat exporting countries and may prevent them from participating in international trade [1].

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COUMAPHOS DISSIPATION, DEGRADATION AND BINDING TO SEDIMENT IN A MODEL LABORATORY VAT SYSTEM USING ^{14}C-LABELLED ACARICIDE

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Abstract

Dissipation and degradation of ^{14}C-labelled coumaphos in suspension and binding to sediment was studied in a model laboratory glass vat was studied. The water in the vat was treated with a mixture of ^{14}C-labelled and unlabelled coumaphos and initial concentration determined. Two weeks later 50 g soil was added to the suspension and 3x25 mL samples taken and analysis. The procedure was repeated every two weeks for 44 weeks. pH was also recorded at each sampling time. At the end of the experiment soil was extracted by using Soxhlet apparatus and by supercritical fluid extraction and concentrations of free and bound coumaphos and metabolites were determined. Very little dissipation of total ^{14}C occurred and 88% of the initial concentration was still present at the end of 44 weeks. The ^{14}C content in the filtrate declined to 20% but there was a corresponding increase in the ^{14}C content of the sediment mixed with emulsion. The pH of the suspension steadily decreased with time. HPLC analysis of the filtrate, soil wash and Soxhlet extract indicated the presence of coumaphos and three metabolites chlorferon, potasan and oxygen analogue of coumaphos. Analysis of supercritical fluid extract of the sediment-bound radioactivity showed the presence of coumaphos but no metabolites. In another study filtered and washed soil from the tank was incubated with an EPTC-degrading bacterial strain, *Rhodococcus* TE1 to study the effect on the metabolism of coumaphos. About 10.9% of ^{14}C was released in the incubation mixture and it contained mostly chlorferon with some oxygen analogue of coumaphos. Exposure of ^{14}C-labelled coumaphos to U.V. and sun lamp, to study photolysis, resulted in the formation of a brown precipitate. While the ^{14}C content in the filtrate decreased, its concentration in the precipitate increased. The filtrate contained primarily coumaphos, whereas, the precipitate contained coumaphos, chlorferon and potasan. These findings suggest that in the field vat the potential for coumaphos degradation and also its precipitation under the sun light conditions may occur.

1. INTRODUCTION

Coumaphos [CO-Ral; 0,0-diethyl 0-(3-Chlor-4-methyl-2-oxo-2H-1-benzopyran-7-yl)phosphorothioate] is used for the control of a number of arthropod pests that attack domestic animals [1]. This acaricide has been used since 1968 in dipping operations and has provided an excellent control of the cattle ticks in the U.S. eradication program [2]. In general, coumaphos concentration level in a vat is considered stable over time and any decrease in the insecticide concentration is normally attributed to removal by cattle. However, losses of coumaphos are possible due to microbial degradation, chemical and photodegradation, and adsorption on sediments which subsequently settle to the bottom of the dipping vats. Therefore, attention is required to monitor the dissipation, degradation and binding to sediment of the acaricide in vats so that no loss of efficacy is experienced.

The purpose of these experiments was to provide information on the dissipation, degradation and binding of ^{14}C-coumaphos in a model laboratory vat system. The experimental protocol established during the first FAO/IAEA Research coordination meeting (1992) was followed in conducting this study.

2. MATERIALS AND METHODS

Chemicals: Co-Ral (coumaphos), A 11.6% emulsifiable formulation was obtained from Miles Inc., Kansas (U.S.A.). Analytical grade coumaphos, its oxygen analogue, chlorferon (3-chloro -

4-methyl-2-oxo-2H-1-banzopyran), potasan [0,0-diethyl-0-(4-methyl-2-oxo-2H-1-benzobyran-7-yl) phosphorothioate] were obtained commercially. Radiolabelled coumaphos (phenyl^{14}C) was a gift from IAEA, Vienna.

Model Vat Solution: A laboratory model glass vat (60x30x30 cm) to accommodate about 42 litres solution was placed in a wooden box. The glass walls were covered with black plastic sheets and the space between the box and the tank was filled with cardboard. To prepare a ^{14}C-homogeneous formulation, cold formulation (106 mL), ^{14}C- coumaphos (3.7 MBq in 10 mL acetone) and 60 mL acetone in an Erlenmeyer flask were magnetic-stirred for about 20 minutes. The homogeneous solution was then quantitatively transferred to the tank and the flask was rinsed with additional amount of acetone (3x10 mL). Tap water was added to the tank to bring the final volume to 41 L and level was marked on the wall of the tank.

Sediment: Soil (50 g, oven - dry - basis) collected from an agricultural field was added to the tank every two weeks.

Sampling: Zero time samples from the tank were taken before the addition of soil. The water in the tank containing ^{14}C-homogenous formulation described above was well mixed and three 1.0 mL samples were collected from a depth of about 15 cm. Soil was then added after initial sampling. Two weeks later water level in the tank was brought to the mark by adding tap water, 50 g soil was added, stirred well for 5 minutes and immediately 3x25 mL samples were collected from a depth of about 15 cm. The pH of the suspension was immediately determined. This procedure was repeated every two weeks till the end of 44 weeks.

Analysis: Samples taken every two weeks were centrifuged or filtered and the ^{14}C content of the supernatant or filtrate was determined. The ^{14}C content of the solid residue was determined by combustion. The solution phase (1 mL) was diluted with methanol (9 mL), shaken vigorously and the supernatent was analyzed by HPLC.

A bulk sample collected at the end of 44 weeks (1.0 L) was filtered under suction through a filter paper and exhaustively washed with distilled water. The filtrate and washings were combined and concentrated to dryness in a rotary evaporator. The material was dissolved in methanol, radioassayed and analyzed by HPLC. The soil residue was extracted with methanol in a Soxhlet extractor for 24 h. The extract was radioassayed and analyzed by HPLC. The soil residue containing bound or nonextractable ^{14}C was subjected to supercritical fluid extraction (SFE) and the released material was analyzed by HPLC (Fig.1).

Biodegradation Study : An EPTC-degrading bacterial strain, *Rhodocossus* TE1, isolated from EPTC-adapted soil[3] was used. An aliquot of the washed soil from sample taken after 44 weeks described above was taken into a sterile Erlenmyer flask and BMNG medium was added [3]. The medium was inoculated with TE1 cells. Aliquots were withdrawn from the incubation mixture at various time intervals for measurement of cell growth, determined spectrophotometrically at 600 nm, and for analysis to determine the metabolic products. Each time the incubation mixture was exhaustively extracted with methanol, radioassayed for ^{14}C and analyzed by HPLC.

Photolysis Study : The photochemical reaction vessels were equipped with an outer water-cooled immersion wells to maintain a constant solution temperature (22°C) during the photolysis reaction. For UV irradiation the light source was a 450 Watt immersion ultraviolet lamp mounted in a 250 mL (water cooled) glass cell and connected to an "Ace-Hanovia" Model 7830 power supply. For sunlight irradiation a 150 Watt xenon compact arc lamp PTI Model A1010

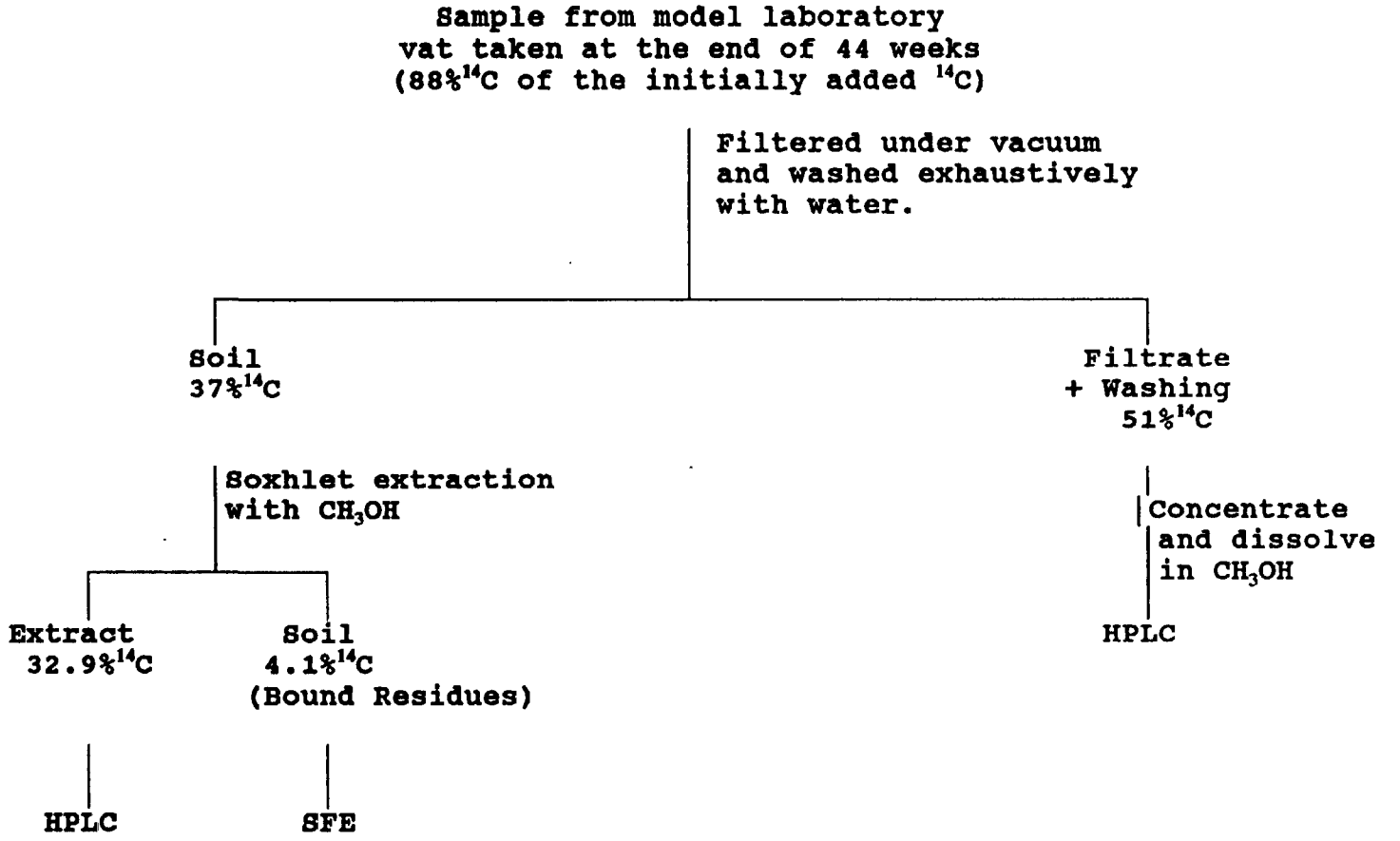

Figure 1. Schematic diagram for the Analysis of sample collected after 44 weeks from the model laboratory vat.

connected to PTI Model -220/250 power supply was used. ^{14}C-homogenous formulation of coumaphos (300 μg/mL) solution (250 mL) described earlier was irradiated till the concentration of ^{14}C-coumaphos was reduced to about 30% of the initial concentration. During the irradiation, a magnetic stirring bar was used to continuously mix the solution. Samples were withdrawn initially and at appropriate intervals and radioassayed. The mixture was filtered and the filtrate was analyzed. The precipitate was dissolved in methylene chloride and analyzed by HPLC.

Determination of Radioactivity : Liquid sample were assayed in a Packard Model 3320 scintillation spectrometer, and sediment were assayed by combustion in a Packard sample oxidizer, Model 307 followed by liquid scintillation counting (LSC) with an external standard and correcting data for quenching.

High-Pressure Liquid chromatography (HPLC) : The high-pressure liquid chromatograph was a Varian LC 5500 equipped with a variable wavelength UV detector (λ=220 nm) connected to a radioactivity monitor and data station (Berthold LB504 and 512). The column was a Whatman Partisil-10 ODS-2 (25 cm long, 9.4 mm inner diameter). The composition of the mobile phase was 30:70, water: methanol. The flow rate was maintained at 1.5 mL/min. Under these experimental conditions the retention times of chlorferon, coumaphos oxygen analogue, potasan and coumaphos were 3.2, 4.0, 5.5 and 7.1 min., respectively.

Thin-Layer Chromatography (TLC) : The concentrated solution were chromatographed for cleanup on preparative silica gel plates (Whatman PLK5F, 20x20 cm, 1000/μm thickness) with a solvent system of toluene-ethanol-acetic acid (75:25:1). The concentrated solution was directly applied at the bottom of the central linear region of the TLC plate while vertical channels on the two sides were used for reference authentic compounds. After development of the plate, it was photographed by a Berthold Beta camera LB292 to detect radioactive regions. The radioactive region at Rf 0.90, 0.40 and 0.10 were scraped off the plate, extracted with methanol, the extracts were radioassayed and finally analyzed by HPLC.

Supercritical Fluid Extraction (SFE) : The supercritical fluid extractor (Suprex Model SFE-50, Suprex corp., Pittsburgh, PA) was used as described by Khan et al [4]. The extraction of soil containing bound ^{14}C was carried out for 3 h at a pressure of 370 atm and temperature of 180°C using carbon dioxide as a supercritical fluid and methanol as a modifier. The flow rates of CO_2 and methanol were maintained at 1.0 and 0.5 mL/min, respectively. The extracted material was radioassayed and analyzed by HPLC.

3. RESULTS AND DISCUSSION

Laboratory vat solution was examined with respect to ^{14}C - coumaphos dissipation. As demonstrated in Fig.2, very little dissipation of total ^{14}C occurred in the well-mixed suspension/emulsion samples taken during the course of the experiment. Thus, at the end of 44 weeks period about 88% of the initially added ^{14}C was still present in the sample collected after stirring the suspension in the tank (Fig.1). There was decline in the initial concentration of ^{14}C in the filtrate over the period of 44 weeks (Fig.2). This in turn corresponded to an increase in the ^{14}C content of the sediment mixed with emulsion. Thus, by the end of the experiment, the ^{14}C concentration in the filtrate decreased to about 20% of the initially added ^{14}C The filtrate obtained under the experimental conditions described showed a decline with time in coumaphos concentration (Fig.3). At the end of 44 weeks period the concentration of coumaphos in the filtrate was found to be about 20 mg/L. A decrease in pH of the mixture with increasing time was also observed (Fig.3). At the end of the experiment exhaustive washing of the sediment

mixed with emulsion removed about 51% ^{14}C while about 37% of ^{14}C remained adsorbed on the sediment (Fig.1). HPLC analysis of the concentrated combined filtrate and washing solution revealed the presence of coumaphos (139.1 mg/L), chlorferon (4.7 mg/L), potasan (4.9 mg/L) and oxygen analogue of coumaphos (0.9 mg/L). Fig. 4 shows the structures of these compounds. Soxhlet extract of the soil contained 32.9% of the initially added ^{14}C while 4.1% ^{14}C remained nonextractable (bound residues) from the soil (Fig. 1). Analysis of the extract by HPLC showed the presence of coumaphos (77.3 mg/L), chlorferon (4.0 mg/L), potasan (3.0 mg/L) and oxygen analogue of coumaphos (1.1 mg/L).

The sediment material containing bound ^{14}C was subjected to supercritical fluid extraction. This resulted in a recovery of 67.4% of bound ^{14}C. Analysis of the released material in the SFE extract by HPLC showed that it contained 6.1 mg/L of coumaphos. No other known metabolite of coumaphos was present in the material released by SFE.

The filtered and washed soil after 44 weeks described earlier was incubated in BMNG medium with *Rhodococcus* TEI. No inhibition on the growth of bacteria was observed during the course of incubation. At the end of nine weeks incubation about 10.9% ^{14}C was released in the incubation mixture. The released material constituted chlorferon (7.4%) and oxygen analogue of coumaphos (2.0%) while 1.5% ^{14}C material remained unidentified.

The results of the photolysis of ^{14}C-emulsifiable formulation of coumaphos with increasing time of exposure to U.V. and visible radiation are shown in Figs 5 & 6. During the course of photolysis under the UV and sun lamps formation of a brown precipitate was observed and its amount increased with increasing time of exposure. The precipitate was filtered from the samples withdrawn at appropriate intervals and the filtrate was analyzed by HPLC for coumaphos concentration. It was observed that while total ^{14}C content of the mixture during photolysis remained nearly constant, the concentration of coumaphos in the filtrate decreased steadily with increasing time (Figs 5 & 6). Thus, at the end of experiment it was observed that the precipitate formed from the emulsifiable formulation of coumaphos exposed to UV (72 h) and sun (130 h) lamps contained 86.5% ^{14}C and 64.5%^{14}C, respectively of the initially added ^{14}C in the formulation. The corresponding values for the filtrate were 8.9%^{14}C and 31.3%^{14}C, respectively. Analyses of filtrate revealed the presence of coumaphos only. The precipitate was dissolved in methylene chloride, subjected to preparative TLC and finally analyzed by HPLC. In both cases coumaphos was partially photodecomposed into two products namely chlorferon and potasan. Thus, the total amount of ^{14}C associated with the precipitate obtained by UV irradiation constituted 71% ^{14}C coumaphos, 23% ^{14}C chlorferon and 6% ^{14}C potasan. The corresponding amounts of ^{14}C associated with the precipitate obtained by sun lamp exposure were 75% ^{14}C coumaphos, 14%^{14}C chlorferon and 11% ^{14}C potasan. It appears that during the photolysis period a considerable amount of coumaphos and its products, chlorferon and potasan, co-precipitated with the matrix material of the emulsion. These observations suggest that in the field vat possesses the potential for coumaphos degradation and also its precipitation under the sun light conditions may occur.

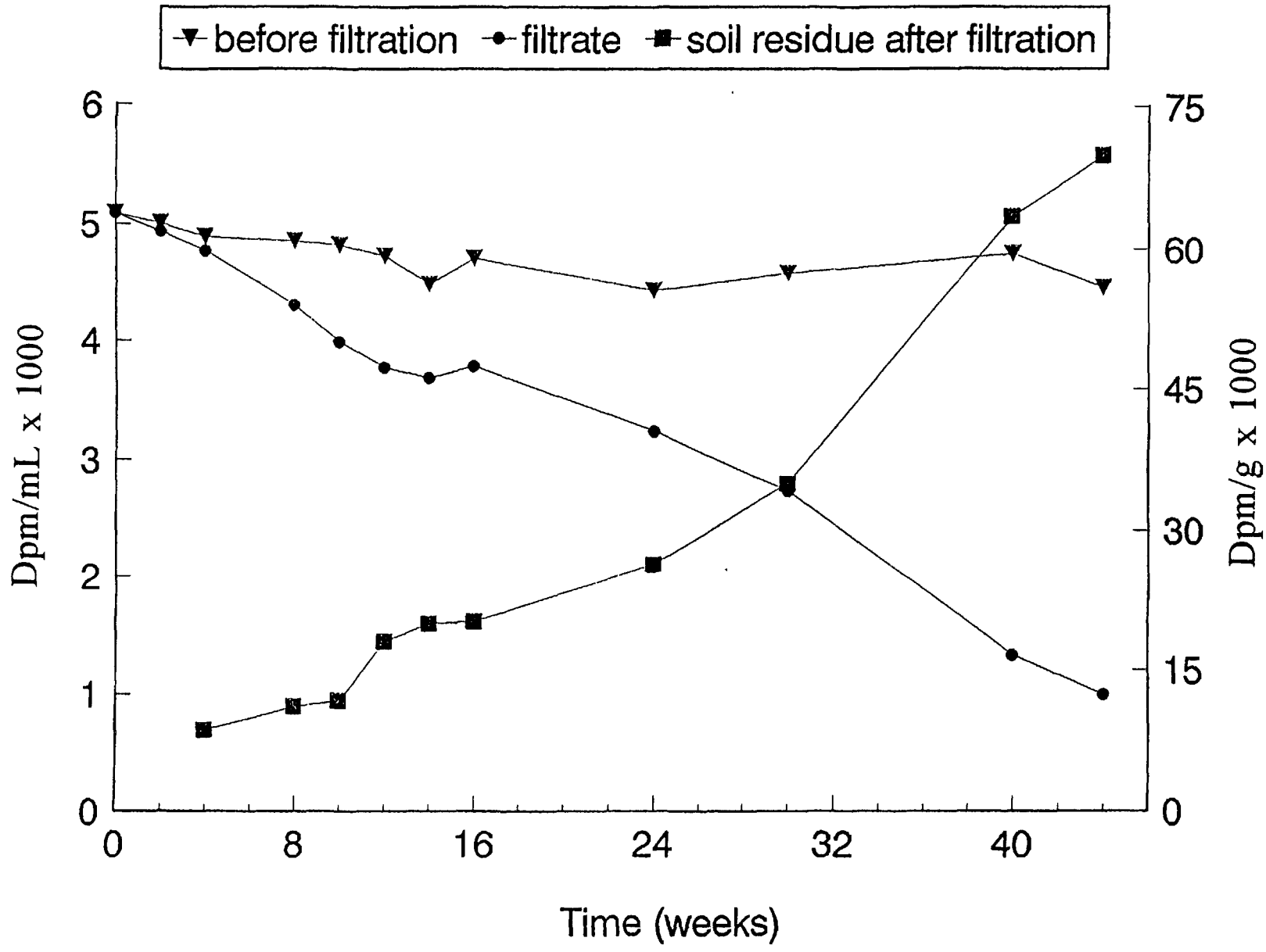

Figure 2. Variation in ^{14}C concentration with time in the model laboratory vat.

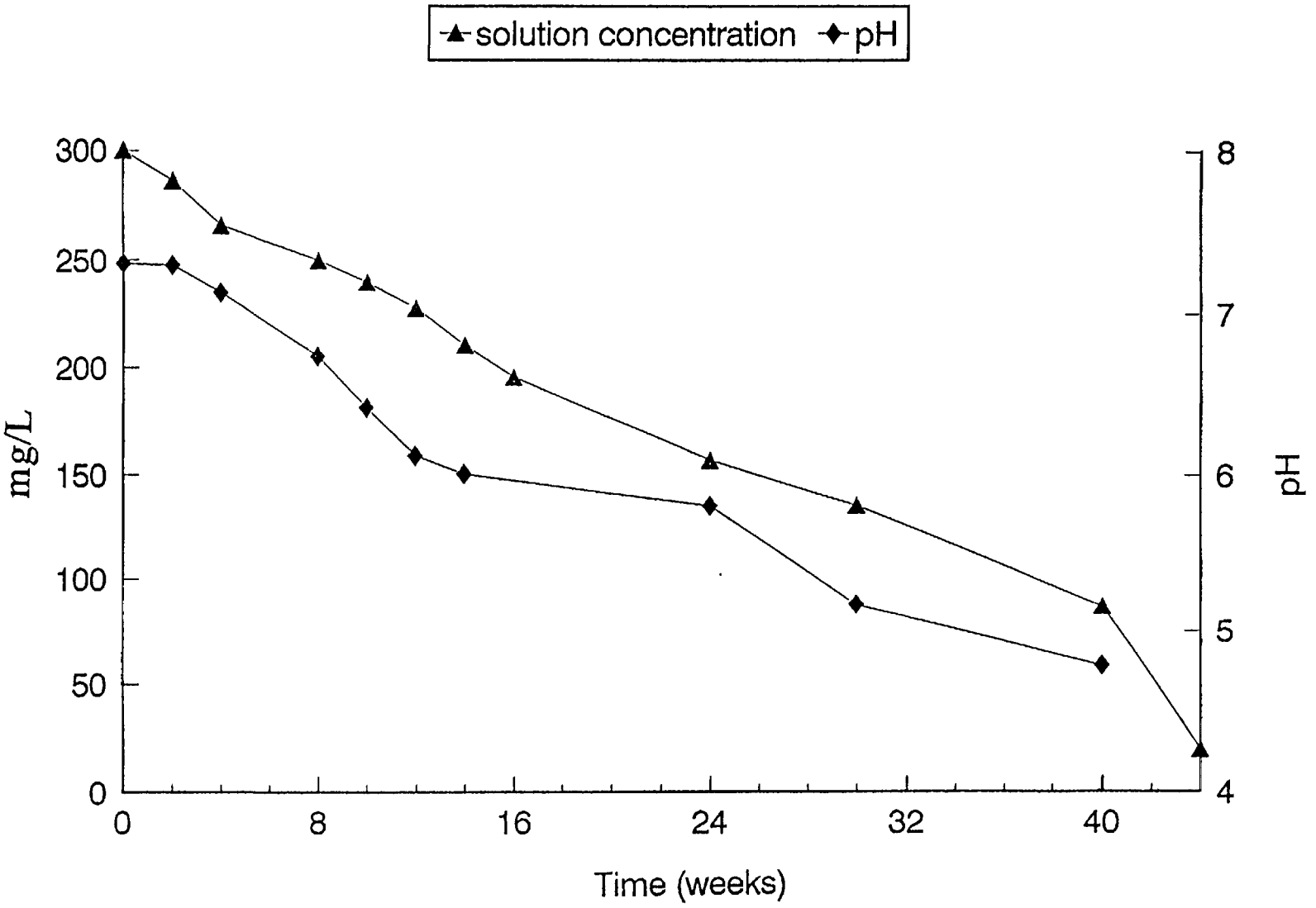

Figure 3. Variation in coumaphos concentration and pH of the solution in the model laboratory vat.

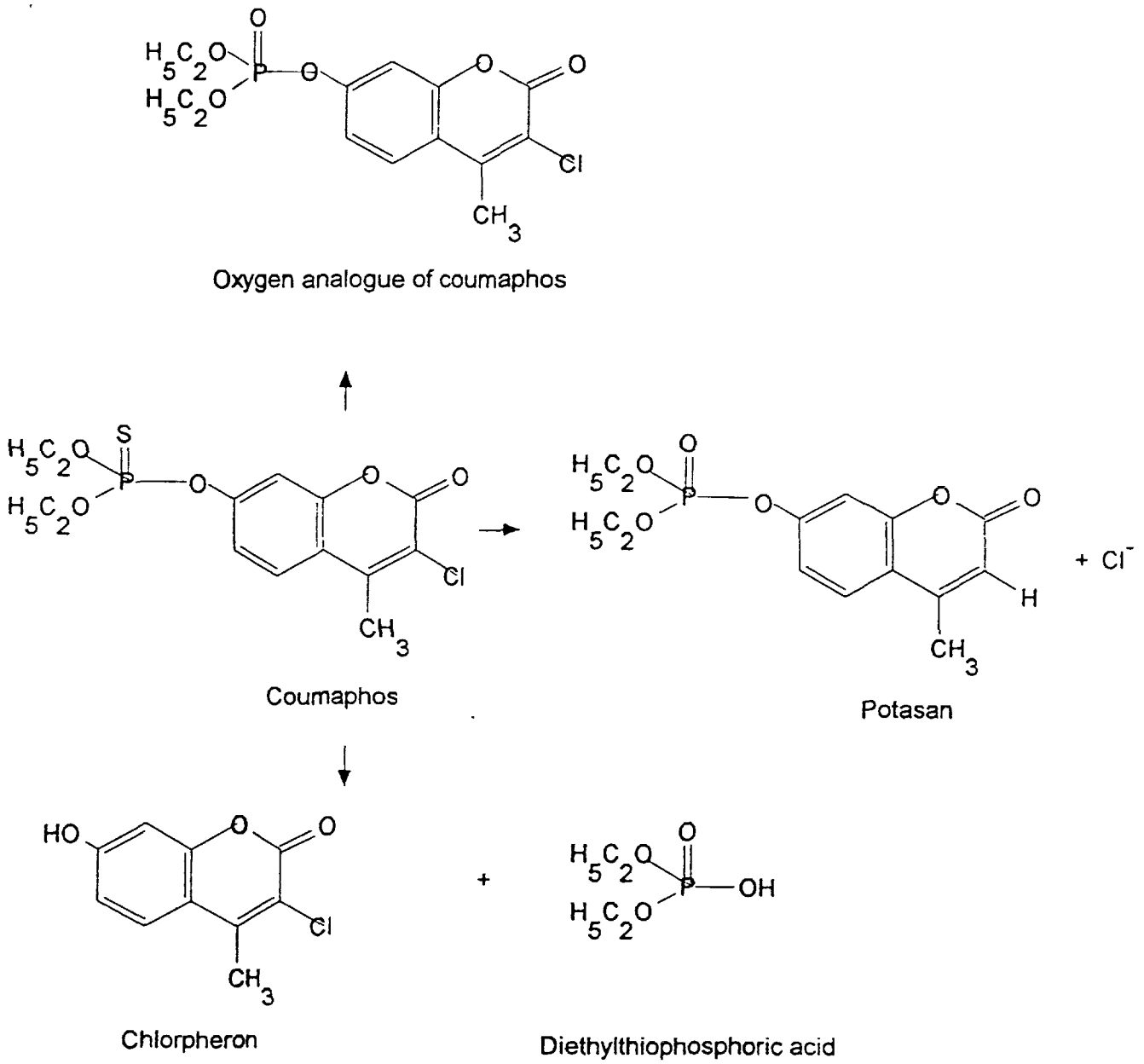

Figure 4. Products formed from coumaphos.

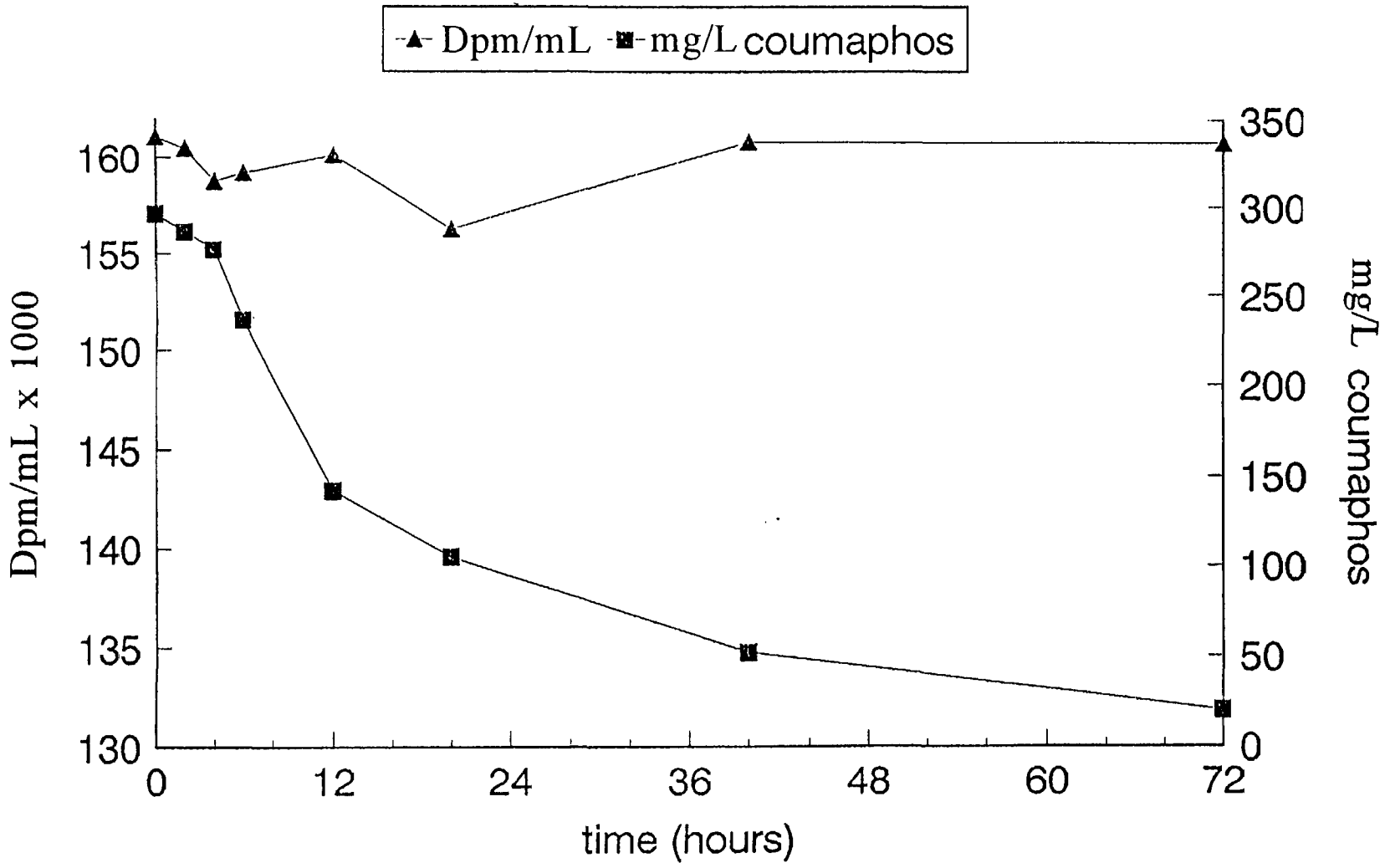

Figure 5. Effect of UV irradiation on ^{14}C-coumaphos.

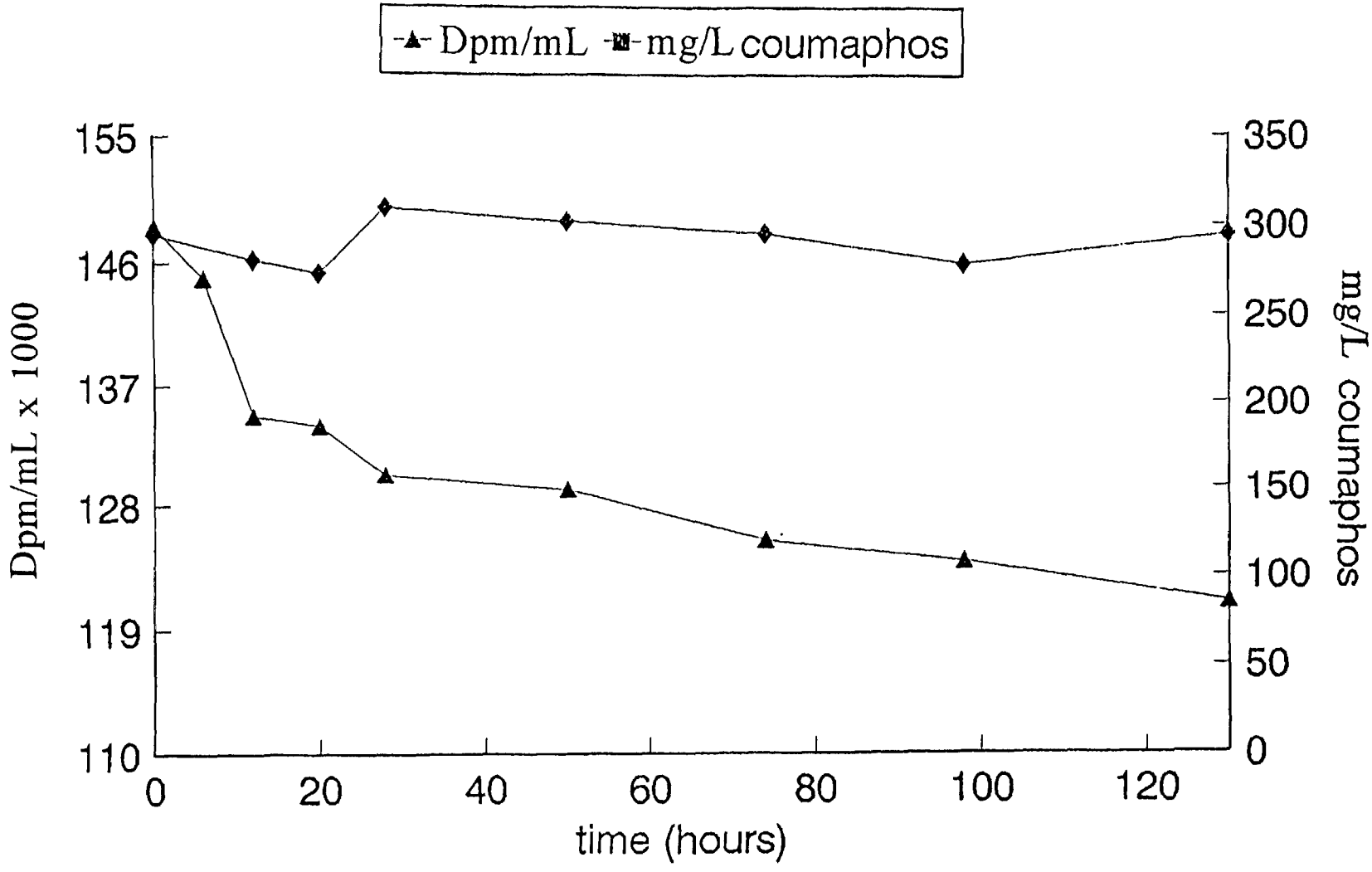

Figure 6. Effect of sun lamp irradiation on ^{14}C-coumaphos.

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DISSIPATION OF COUMAPHOS ACARICIDE IN MODEL CATTLE DIPPING VATS AND SOIL COLUMNS UNDER SUB-TROPICAL CLIMATE OF DELHI

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Abstract

The stability of coumaphos was studied in model dipping vats under field conditions using ^{14}C-labelled and unlabelled coumaphos, with or without additives. Four vats were used each containing 50 litre of water treated with 3.7 MBq ^{14}C and 10 g (AI) unlabelled coumaphos in 25 mL acetone Vat 1 was control. Vat 2 and 3 were maintained at pH 5 by addition of superphosphate initially and at regular intervals as required. Vat 3 and 4 were treated with bacteriostat copper sulphate at 0.01 g per litre. The pH of vat 1 and 4 gradually increased with time from initial value of 7 to 8.47 and 7.57 respectively. In vat 1 and 4 the concentration of coumaphos declined from about initial concentration of 200 μg/mL to about 9 μg/mL in 255 days after initial treatment, whereas the persistence of coumaphos was more in vats 2 and 3. The stability of coumaphos in model vats increased significantly by maintaining a pH of 5 by addition of superphosphate. The pesticide residues consisted of 80 % or more of unchanged coumaphos. In addition potasan, chlorferon and 4 - methylumbelliferone were detected in small proportions. In another experiment the effect of pH (4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8 and 8.5) was studied. Five litre buffer in each glass jar was treated with 74 kBq ^{14}C and 1 g (AI) unlabelled coumaphos in 20 ml acetone. Coumaphos was most stable at pH 5 as also observed in model vats under field conditions. Leaching, degradation and dissipation of coumaphos, alkylated coumaphos and aged residues of vat 2 and 3 were studied in soil columns in PVC pipes in the field. Coumaphos did not leach below 10 cm in all the four cases. Dissipation, degradation and bound residue formation was more in case of alkali treated coumaphos than normal coumaphos. Aged residues of vat 3 were reasonably stable as copper sulphate inhibited the degradation of coumaphos in soil by microorganisms. Coumaphos along with the the above three metabolites were detected in the extractable insecticide residues from the soil, though the proportion of metabolites was much more than found in model vats. Again treatment of coumaphos with alkali further increased its metabolism and the soil bound residues were double as compared to untreated coumaphos.

1. INTRODUCTION

Coumaphos [O , O - diethyl O - (3 - chlor - 4 - methyl - 2 - oxo - 2H - 1 - benzopyran - 7 - yl) phosphorothioate] is an organophosphorus insecticide/ acaricide used in cattle vats for the control of southern cattle ticks, *Boophilus microplus* Say. and *Boophilus annulatus* Can. The maintenance of a homogenous solution of coumaphos with adequate concentration in cattle vats is important for effective control and to avoid development of resistance in ectoparasite. Loss of coumaphos affects its biological and acaricidal activity. This requires regular monitoring and development of procedures to improve stability [1]. Coumaphos degrades anaerobically and aerobically in highly used vats. In aged vats coumaphos degrades by reductive dechlorination under anaerobic conditions to potasan [2]. However, the degradation can be controlled by the use of biological agents viz., 2,4-benzis, dichlorphen, copper sulfate, etc. Microbial metabolism of coumaphos is rapid and results in cleavage of phosphorothioate linkage to yield chlorferon [3]. They further reported that the waste liquids generated from the used vats are difficult to degrade in soil disposal due to persistence of coumaphos. Waggoner [4] reported the half life of coumaphos in sandy loam soil under laboratory conditions to be about 300 d.

Biodegradation of coumaphos in cattle vats may cause loss of bioefficacy of pesticides. Karns et al [2] suggested that at pH 5.0 the degradation of coumaphos may be controlled to a large extent, presumably due to an inhibition of the microbial activity . Degradation of pesticide can be further prevented by addition of bactericides. Shelton and Karns [5] also reported that degradation of coumaphos is inhibited due to decrease in pH.

A field experiment was undertaken to study the dissipation and degradation of coumaphos in four different model vats and simultaneously laboratory studies were undertaken on the dissipation of coumaphos at different pH under field conditions.

Coumaphos waste are dumped in the soil evaporation pits, ditches or ponds most of which are unlined. As a result, the underlying soils are contaminated with high concentration of coumaphos and its metabolites. This may further contaminate the surface and ground water by surface run-off or leaching. Field experiments were also conducted to determine the degradation, dissipation and leaching of aged coumaphos residues in comparison to fresh coumaphos in soil columns. The purpose of this study was to provide information on the dissipation, degradation and binding of ^{14}C-coumaphos in model vats and to evaluate the stability of coumaphos in the presence of a buffer and a bacteriostat and also to know the leaching, degradation and dissipation of coumaphos and aged residues with or without buffer and bacteriostate in soil columns.

2. MATERIALS AND METHODS

Four small scale models of cattle dipping vats of dimension 100 x 38 x 100 cm were constructed outdoors in the Department of Zoology, University of Delhi, with bricks and plastered with cement. They were filled with 50 L water each. Each vat was treated with 3.7 MBq ^{14}C and 10 g (AI) unlabelled coumaphos in 25 mL acetone. Vat 1 was control. In vat 2 and 3 pH was adjusted to 5.0 by adding single super phosphate at 10 g per litre. In vat 3 and 4 copper sulphate was added as bacteriostat at 0.01 g/L. In vat 1 and 4 the initial pH was 7.0. In vat 2 and 3 pH was maintained at 5.0 by adding super phosphate at regular intervals. Before sampling the volume of water was made up to the mark by adding tap water in each vat and then they were mixed vigorously. Sampling periods are shown in Table I. Triplicate samples of 250 mL were taken from each vat. Three aliquots of 1mL each was taken from each sample in a scintillation vial and 10 mL of scintillation cocktail Amersham BCS 104 was added and mixed and radioactivity estimated by Packard 2000 CA Scintillation Spectrometer. Fifty mL of each sample was partitioned with 20 mL of methylene chloride in separatory funnel and this was repeated three times. Pooled methylene chloride extracts were concentrated to 0.5 mL in a Buchii flash evaporator. Cleanup was carried out by column chromatography - on 10 cm high column of activated silica gel (2% hydrated). Concentrated sample was diluted with 5 mL eluting solvent and poured onto the column and the column was eluted with 25 mL solution of eluting solvent- 25% acetone in methylene chloride at a flow rate of 0.15 mL/min. Effluent was collected and concentrated to 1 mL. For TLC analysis 500 µL of sample was spotted on preactivated Baker flex Silica gel IB 2-F TLC plate (60 ^{0}C for one hour). Mobile phase was ethyl acetate : toluene : heptane (45:50:15 v/v) and spots were visualized by UV. Coumaphos and its metabolites potasan and chlorferon were identified by standards and their Rf values (0.96,0.63, and 0.55 respectively). HPLC was carried out with Shimadzu LC-4A on Whatman partisil -10 ODS - 2 column (9.6 cm long, 250 mm dia). Mobile phase was water : methanol (30:70 ,v/v). The flow rate was 1.5mL/min and detector wavelength used was 220 nm. Retention times of standards, 4 - methyl umbelliferone, chlorferon, potasan and coumaphos were 2.2, 2.6, 5.0 & 6.5 min respectively (Fig 1). In model vats after 7 months 1 Kg mixture of soil and cowdung (1:1) was added. Ten litre water was taken out of each vat with vigorous shaking after 16 months. Sediment was collected, air dried and amount was weighed. Total, extractable and bound residues were estimated.

In another experiment the effect of pH (4.5 to 8.5) was studied. Nine glass jars of 7 L capacity were half immersed in soil and were covered with wire mash and then with black polyethylene sheet to avoid photodegradation. Jars were filled with 5 L buffer of particular pH.

TABLE I. THE EFFECT OF ADDITIVES ON THE DISSIPATION OF COUMAPHOS IN MODEL CATTLE DIPPING VATS.

Days after treatment	Radioactivity remaining in vat suspension (dpm/mL)			
	Vat No. 1[a]	Vat No. 2[b]	Vat No. 3[c]	Vat No. 4[d]
0	4415±42	4395±14	4449±81	4655±19
15	4284±208	4387±326	4356±28	4324±92
30	4173±81	4324±81	4258±342	4213±87
45	4022±117	4267±110	4142±287	4058±217
60	3897±201	4200±29	4031±75	3906±86
75	3733±39	4017±117	3925±49	3395±29
105	3412±79	3906±39	3893±49	3250±22
135	2717±106	3625±76	1779±68	2744±37
165	1622±10	2845±125	2895±59	1918±183
195	461±19	1837±53	2301±29	304±24
225	271±15	1477±31	1466±36	230±7
255	209±37	1565±131	1185±86	199±8

[a] Vat No. 1 contained 10 g mixture of ^{14}C-labelled (3.7 MBq) and unlabelled coumaphos in 50 L water as suspension at pH 7 and was used as control.
[b] Vat No. 2 contained coumaphos suspension in water + superphosphate fertilizer (10 g/L) as buffer; initial pH adjusted at 5.
[c] Vat No. 3 contained coumaphos suspension in water + superphosphate + copper sulfate (0.01 g/L); initial pH adjusted at 5.
[d] Vat No. 4 contained coumaphos suspension in water + copper sulfate; initial pH was 7.

Each jar was treated with 74 kBq ^{14}C and 1 g (AI) unlabelled coumaphos in 20 ml acetone. Thirty ml samples in triplicate were taken after vigorous shaking and maintaining pH of the solution. Extraction and analysis were carried out as above.

Leaching, degradation and dissipation of coumaphos, alkylated coumaphos and aged residues from vats 2 and 3 were studied in soil columns. Polyvinyl chloride (PVC) pipes open at both ends (60 cm x 5 cm i.d.) were pushed into soil. A 10 cm rim of pipes were left above the ground to prevent run off water from washing away the soil in columns. The PVC pipes were left undisturbed for equilibration for two months. Four sets of PVC pipes were used . In first set each soil column was treated with 74 kBq and 4 mg unlabelled coumaphos in 5 mL water. In second set 74 kBq and 4 mg unlabelled coumaphos in 5 mL 0.2 N KOH solution kept for 24 hours prior to treatment was added to each column. In third and fourth set of PVC pipes, soil

columns were treated with extracts of vat 2 and 3. Pipes were taken out of the soil at definite intervals and were divided in 5 fractions of 10 cm soil each. Soil from each fraction was taken out carefully and air dried with frequent stirring and then was grinded in mortar and pestle to fine powder. Triplicate soil samples of 300 mg each were combusted in Harvey biological oxidizer and radioactivity was estimated for each fraction. Fifty gm samples in triplicate from soil containing radioactivity were extracted with 150 mL methanol in soxhlet for 24 hours. Extractable residues were estimated by the radioactivity present. 300 mg extracted soil samples in triplicate were combusted in tissue oxidizer and bound residues were estimated. Methanol extracts were cleaned and TLC and HPLC was carried out as described earlier.

3. RESULTS AND DISCUSSION

3.1. Persistence and metabolism of coumaphos in model vats

Each vat was filled with 50 L of water and four vats were used for the experiment and each vat was filled with 50 L of water. Vat 1 was taken as control and Vat 2 to 4 were experimental. Each vat was treated with 3.7 MBq ^{14}C and 10 g unlabelled coumaphos. At zero time the radioactivity estimated in vats as dpm/ml was 4415, 4395, 4449 and 4655 respectively. This declined to 209, 1565, 1185 and 199 dpm/ml in 255 days after the treatment. It further declined to 97, 351, 280 and 98 dpm/ml in 285 days after the treatment and this may partly be attributed to heavy rainfall and over flooding of the vats after 255 days of the sampling. At zero time these values were 199, 198, 200 and 210 for vats 1 to 4 respectively. This declined to 9, 71, 53 and 9 in 255 days after the treatment. The dissipation of coumaphos was comparatively slow in vat 2 and vat 3 in comparison to vat 1 and vat 4. The half lives of coumaphos in vats 1 to 4 were 193, 486, 573 and 177 d up to 135 d while overall half lives of coumaphos upto 255

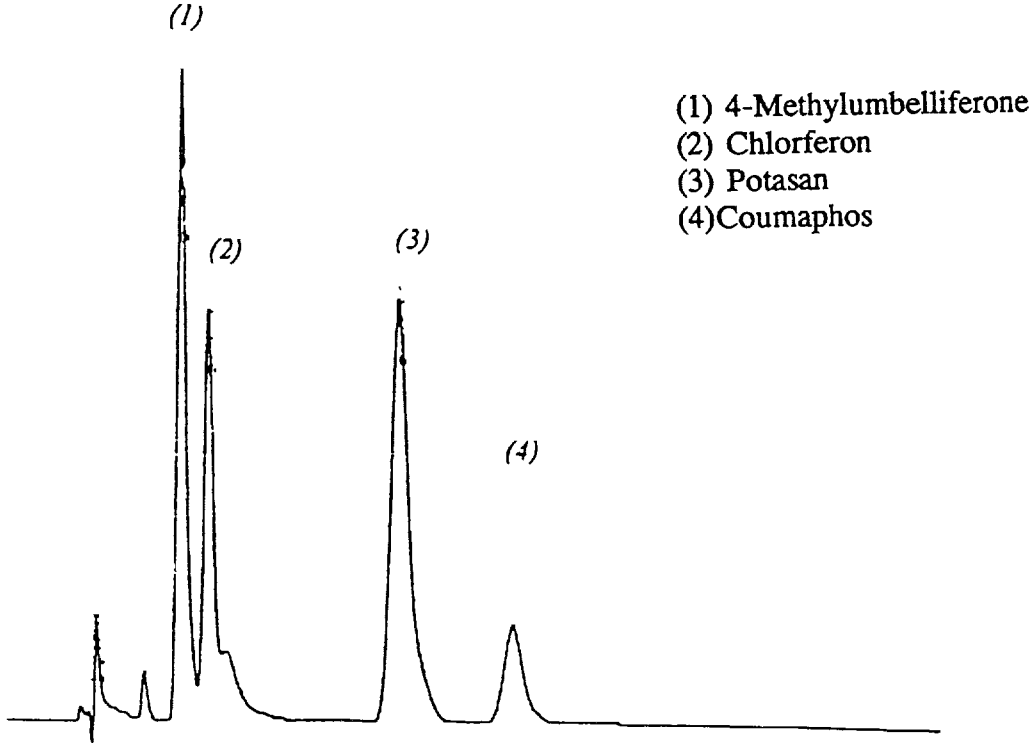

FIGURE 1. HPLC CHROMATOGRAM OF COUMAPHOS AND METABOLITES.

d were 58, 172, 134 and 56 d, respectively. After analysing the data it can be concluded that dissipation was relatively slow at pH 5.0 in vat 2 & 3 which contains both buffer and copper sulfate(vat 3) as bactericide. The dissipation of pesticide was rapid in vat 1 and vat 4 . The microbial degradation affects the efficacy of coumaphos. Shelton et al [6] reported that microbial consortia degraded the pesticide effectively. By reducing the pH to 5.0, the degradation of organophosphate acaricide can be minimized. Presumably it inhibits the microbial activity. However, the breakdown of the acaricide in vats is presumed to be further prevented by addition of bactericides viz. $CuSO_4$, $MgCl_2$ etc. However, in the present study there is very little difference between the vat 2 and 3, thereby showing that copper sulfate does not seem to have any additional effect. Analysis of sediment collected after 16 months from vat 1 and 4 shows that concentration of pesticide was more in vat 4 sediment i,e. 72034 dpm per g as compared to 34473 dpm per g in vat 1 sediment. Copper sulphate, a heavy metal compound, caused rapid settling of pesticide from suspension by breaking the emulsion [7]. The variation in pH in vats throughout the experimental period is given in Table II. The composition of the insecticide residues in the vats was analysed by TLC and HPLC. The major metabolites of coumaphos and their resolution by HPLC is shown in Fig. 1. The samples showed the presence of four main peaks which were identified to be 4-methylumbelliferone, chlorferone, potasan and coumaphos according to their

TABLE II. CHANGE IN THE pH OF THE VATS RECORDED PRIOR TO EACH SAMPLING.

Days after treatment	pH of the suspension			
	Vat No. 1	Vat No. 2	Vat No. 3	Vat No. 4
0	7.1	5.0	5.0	7.0
15	7.0	5.1	5.0	7.0
30	7.6	5.2	5.3	7.1
45	7.7	5.0	5.0	7.3
60	7.9	5.2	5.1	7.2
75	8.1	5.0	5.2	7.3
105	8.2	5.1	5.1	7.3
135	8.6	5.4	5.4	7.3
165	7.5	5.2	5.4	7.3
196	7.9	5.5	5.5	7.5
225	8.0	5.1	5.2	7.5
255	8.5	5.5	5.4	7.6

TABLE III. CONCENTRATION OF COUMAPHOS AND METABOLITES IN VAT NO. 1 AT EACH SAMPLING.

Days after treatment	Concentration of metabolites (µg/mL)[a] 4-Methyl-umbelliferone	Chlorferon	Potasan	Coumaphos
0	—	0.99 ± 0.00	1.88 ± 0.13	186.23 ± 0.32
15	—	1.88 ± 0.00	1.98 ± 0.00	171.92 ± 0.50
30	—	0.65 ± 0.00	4.49 ± 0.05	173.50 ± 0.22
45	—	1.20 ± 0.01	4.87 ± 0.02	180.60 ± 0.27
60	1.63 ± 0.06	2.69 ± 0.14	5.14 ± 0.26	164.44 ± 0.85
75	2.63 ± 0.02	2.17 ± 0.02	4.78 ± 0.05	148.31 ± 0.15
105	3.83 ± 0.07	2.73 ± 0.06	6.04 ± 0.14	136.90 ± 0.32
135	3.16 ± 0.09	2.99 ± 0.12	5.06 ± 0.20	111.91 ± 0.44
165	2.11 ± 0.01	2.31 ± 0.01	2.62 ± 0.02	61.22 ± 0.04
195	0.70 ± 0.02	0.69 ± 0.03	0.95 ± 0.04	15.43 ± 0.06
225	0.44 ± 0.02	0.44 ± 0.02	0.53 ± 0.03	8.78 ± 0.05
255	0.34 ± 0.05	0.34 ± 0.06	0.42 ± 0.08	6.72 ± 0.12

[a] Concentration calculated from radioactivity and reported as coumaphos equivalent

TABLE IV. CONCENTRATION OF COUMAPHOS AND METABOLITES IN VAT NO. 2 AT EACH SAMPLING.

Days after treatment	Concentration of metabolites (µg/mL)[a] 4-Methyl-umbelliferone	Chlorferon	Potasan	Coumaphos
0	—	0.73 ± 0.00	1.87 ± 0.29	191.19 ± 0.14
15	—	0.91 ± 0.00	1.17 ± 0.00	186.27 ± 0.12
30	—	1.02 ± 0.00	0.86 ± 0.00	184.38 ± 0.23
45	—	1.28 ± 0.01	1.11 ± 0.01	189.17 ± 0.66
60	0.20 — 0.00	1.39 ± 0.01	1.33 ± 0.01	185.72 ± 0.13
75	0.31 ± 0.01	1.37 ± 0.04	1.13 ± 0.03	177.12 ± 0.52
105	0.34 ± 0.00	1.48 ± 0.01	1.34 ± 0.01	171.82 ± 0.17
135	0.48 ± 0.01	1.79 ± 0.04	1.45 ± 0.03	158.04 ± 0.34
165	0.48 ± 0.02	1.91 ± 0.08	1.87 ± 0.08	122.33 ± 0.54
195	0.69 ± 0.02	1.51 ± 0.04	1.12 ± 0.03	78.54 ± 0.23
225	0.64 ± 0.01	1.36 ± 0.03	1.03 ± 0.02	62.06 ± 0.13

[a] Concentration calculated from radioactivity and reported as coumaphos equivalent

TABLE V. CONCENTRATION OF COUMAPHOS AND METABOLITES IN VAT NO. 3 AT EACH SAMPLING.

Days after treatment	Concentration of metabolites (µg/mL)[a]			
	4-Methyl-umbelliferone	Chlorferon	Potasan	Coumaphos
15	-	1 52 ± 0 00	3 01 ± 0 00	180 81 ± 0 34
30	-	1 76 ± 0 02	1 05 ± 0 04	167 41 ± 0 75
45	-	1 82 ± 0 00	2 01 ± 0 01	179 97 ± 0 13
60	1 11 ± 0 02	1 87 ± 0 03	2 58 ± 0 05	173 98 ± 0 32
75	1 72 ± 0 02	2 37 ± 0 03	4 38 ± 0 05	165 80 ± 0 21
105	2 70 ± 0 03	3 48 ± 0 04	4 92 ± 0 06	159 88 ± 0 20
135	2 76 ± 0 04	3 91 ± 0 07	3 91 ± 0 07	155 02 ± 0 28
165	2 21 ± 0 03	3 09 ± 0 06	3 05 ± 0 06	118 37 ± 0 24
195	1 46 ± 0 01	2 62 ± 0 03	3 66 ± 0 05	92 94 ± 0 12
225	1 30 ± 0 02	1 87 ± 0 05	1 73 ± 0 04	58 92 ± 0 14
255	1 14 ± 0 06	1 64 ± 0 12	1 22 ± 0 09	47 44 ± 0 34

[a] Concentration calculated from radioactivity and reported as coumaphos equivalent

TABLE VI. CONCENTRATION OF COUMAPHOS AND METABOLITES IN VAT NO. 4 AT EACH SAMPLING.

Days after treatment	Concentration of metabolites (µg/mL)[a]			
	4-Methyl-umbelliferone	Chlorferon	Potasan	Coumaphos
0	-	0 89 ± 0 00	1 55 ± 0 00	188 44 ± 0 39
15	-	1 62 ± 0 00	2 28 ± 0 21	175 40 ± 0 50
30	-	1 20 ± 0 00	4 48 ± 0 10	173 86 ± 0 27
45	-	1 78 ± 0 00	5 26 ± 0 00	176 81 ± 0 11
60	2 26 ± 0 04	2 00 ± 0 04	7 00 ± 0 15	162 56 ± 0 36
75	2 41 ± 0 02	3 78 ± 0 03	6 14 ± 0 05	138 68 ± 0 12
105	2 44 ± 0 01	3 94 ± 0 03	6 16 ± 0 04	129 06 ± 0 09
135	2 45 ± 0 03	3 56 ± 0 05	5 30 ± 0 07	110 37 ± 0 15
165	1 90 ± 0 14	2 67 ± 0 25	2 91 ± 0 28	75 79 ± 0 72
195	0 35 ± 0 02	0 43 ± 0 03	0 39 ± 0 03	11 94 ± 0 09

[a] Concentration calculated from radioactivity and reported as coumaphos equivalent

retention times. The radioactivity was also detected in only these peaks. The composition of coumaphos and its metabolites in the vats is presented in Table III to VI. The maximum degradation was in vat 1. Among metabolites the concentration of chlorferon was more as compared to potasan and 4 methyl umbelliferone. Coumaphos is most stable in vat 2. In vat 4 degradation was less as compared to vat 1 (Tables III & VI), which shows the microbicidal effect of copper sulphate. Coumaphos degrades into the toxic product potasan [O , O - diethyl O - (4 - methyl - 2 - oxo - 2H - 1- benzopyran - 7 - yl) - phophorothioate] , but at lower pH ($\leq$5.5) this degradation process may be eliminated [7], presumably due to inhibition of microbial activity. Under aerobic conditions parathion hydrolase producing bacteria (*Flavobacterium*) [8], cleaves the phosphorothioate linkage of coumaphos and potasan to yeild chlorferon [3 - chloro - 4 - methyl - 2 - oxo - 2H - 1 - benzopyran - 7 - ol] and 4 - methyl umbelliferone respectively. The products of this reaction are no longer acetylcholinesterase inhibitors. α_- Chloro - β - methyl - 2,3,4, - trihydroxy - trans - cinnamic acid (CMTC) and β - methyl - trans - cinnamic acid (MC) are microbial degradation products of chlorferon and 4-methyl umbelliferone [8].

3.2. Effect of pH on coumaphos

In another experiment the effect of pH on the stability of coumaphos was studied and results are presented in Fig. 2. Maximum degradation was observed in alkaline pH. Dissipation is relatively slow in acidic pH around 5.0. Mulbery et al [8] reported that optimum pH for biodegradation of coumaphos was pH 7.5 to pH 8.5. The water from each sample was subjected to TLC and HPLC and it was observed that in acidic pH around 5.0 the degradation of coumaphos is slow. After 240 days the concentration of 4 methyl umbelliferone, chlorferon, potasan and coumaphos in acidic pH 5.0 and alkaline pH 8.5 was 40, 37, 42, 1143 and 84, 108, 90, 1119 dpm/mL, respectively.

3.3. Persistence and leaching of coumaphos

Coumaphos did not leach below 10 cm in 150 day in all four types of columns. Dissipation of coumaphos in soil is very slow. It declined from 19426 dpm/g soil at zero day to 17962 dpm/g soil in 150 days after treatment. The data of total, extractable and bound residue in µg/g soil for coumaphos, alkali treated coumaphos and aged residues are presented in Table VII. Dissipation of coumaphos was more in alkali treated coumaphos 87.6% as compared to normal coumaphos 96.1%. Formation of soil-bound residues was significant in alkali treated coumaphos 21.7% in comparison to normal coumaphos 10.3 %. Extractable residues were subjected to TLC and HPLC analyses and it was observed that degradation was more in alkali treated coumaphos as compared to fresh coumaphos. In case of aged residues of vat 2 and 3 the bound residues were more in the later 6.9% than former 3.1%. Degradation of aged residues of vat 3 was significantly less than aged residues of vat 2. After 150 days the ratio of 4 methyl umbelliferone, chlorferon, potasan and coumaphos in vat 2 and vat 3 aged residues in soil was 19.4 : 11.7 : 20.7 : 48.2 and 4.5 : 3.7 : 4.9 : 86.9, respectively. In soil copper sulphate slowed down the degradation of coumophos because of its biocidal property. Copper sulphate, a heavy metal compound may cause problem in disposal because of the potential adverse environmental impact of heavy metals in the soil [7].

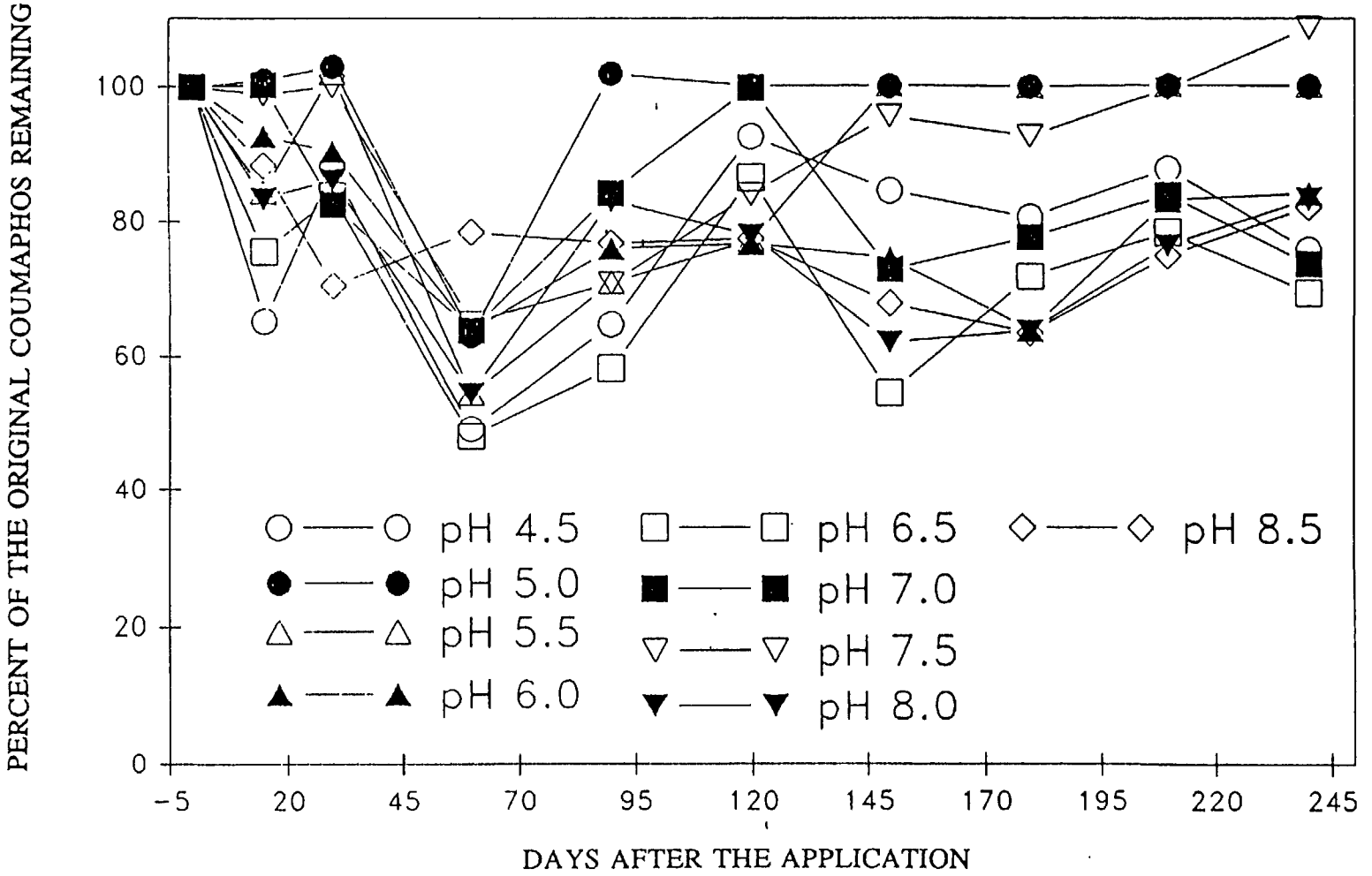

FIGURE 2. DISSIPATION OF COUMAPHOS IN AQUEOUS SUSPENSION IN GLASS JARS UNDER DIFFERENT CONDITIONS OF pH.

TABLE VII. RESIDUES OF COUMAPHOS DETERMINED IN SOIL COULMNS TREATED WITH ALKALI-PRETREATED AND UNTREATED COUMAPHOS.

		Concentration of coumaphos residues in soil (μg/g)		
Type of treatment	Days after treatment	Total residues	Extractable residues	Bound residues
Alkali-treated coumaphos	0	19.52±0.21	17.03±0.10	2.40±0.02
	7	18.51±0.11	16.01±0.04	2.49±0.01
	15	18.44±0.15	15.89±0.08	2.53±0.04
	30	18.37±0.04	15.79±0.03	2.57±0.01
	60	18.32±0.11	15.49±0.09	2.60±0.03
	150	17.06±0.09	13.32±0.02	3.70±0.08
Untreated coumaphos	0	18.81±0.34	17.73±0.09	0.80±0.01
	7	18.53±0.17	17.65±0.08	0.85±0.02
	15	18.31±0.12	17.40±0.11	0.89±0.01
	30	18.12±0.10	17.18±0.07	0.92±0.02
	60	17.96±0.12	16.92±0.11	0.96±0.05
	150	17.39±0.31	15.54±0.17	1.79±0.03

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PERSISTENCE OF LINDANE IN MODEL CATTLE DIPS IN SUB-TROPICAL CLIMATE OF DELHI, INDIA

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Abstract

Persistence of lindane in model cattle dips under field conditions in the sub-tropical climate of Delhi was studied. In one set of experiments two model dips were utilized and filled with 200 L of water and treated with 2.37 MBq of ^{14}C-labelled and 4 g unlabelled lindane each. The radioactivity in water samples at zero time was estimated and considered to be 100 % (744 dpm /mL) which declined to 17.5 % (132 dpm/mL) 180 d after the treatment in Dip 1 (control dip) In the second dip 50 g of cowdung and soil were added 28 d after treatment and subsequently every time before sampling. The results in both the dips were similar indicating no effect of soil and cowdung on the persistence of lindane in the dips. The half life of lindane in water was 101 days. Lindane was extracted from water by hexane in the presence of methanol. The efficiency of this procedure was 99+ % . The main metabolites of lindane in water, identified by HPLC, were 1, 2, 4- trichlorobenzene, 1,3,5- trichlorobenzene and an unknown compound.

In another experiment, 50 L of water was added to one dip which was treated with 2.90 MBq of ^{14}C-labelled and 1 g unlabelled lindane in 25 ml acetone. The cattle dip was recharged at monthly intervals with about 10 % of the initially applied lindane (290 kBq of ^{14}C labelled and 100 mg unlabelled lindane in 25 mL acetone). Soil and cow dung were added as in the first experiment. The concentration of lindane declined rapidly to 11.69 % (1.9 µg/mL, 425 dpm/mL) after the third recharge. The main metabolite was 1,2,4-trichlorobenzene which accounted for about 21 % after first and second recharge. The effect of pH ranging from 5 to 9 was also investigated. The dissipation appeared to be faster at pH 8 and comparatively slower at pH 6.

1. INTRODUCTION

It has been observed that wherever cattle dips are used to control the pests especially ticks of cattle, sheep, goats, etc., the pests tend to develop resistance against the acaricides [1]. This is due to a decline in the concentration of the acaricide in the dips with time thereby increasing the survival rate of the pests and hence the development of resistance. Therefore, a study on the persistence of the acaricide under local climatic conditions and development of methods to stabilize the acaricides in the dips is of utmost importance. However , before this can be attempted, a knowledge of the causes of this decline in the concentration of acaricides is essential. Lindane is one such compound which has been in use since some time as an acaricide [2]. Hence, such studies were undertaken with lindane using radioactive and unlabeled compounds.

2. MATERIALS AND METHODS

Small scale models of cattle dips were constructed outdoors with bricks and plastered with cement (100 cm x 38 cm x 100 cm). For the first experiment these were filled with 200 L of water. One was kept as control (Dip 1) and second as the experimental dip (Dip 2). Each dip was treated with 2.46 MBq ^{14}C (sp. act. 647.5 MBq/mmol) and 4 g unlabeled lindane in 25 mL acetone. Cowdung and soil mixture (100 g 1:1, w/w) was added to the experimental dip every time before removing the sample after the initial addition of cowdung 28 d after treatment with lindane. Each sample of 50 mL was replicated three times from each dip. The experiment was continued for one year and sampling periods are given in Table I.

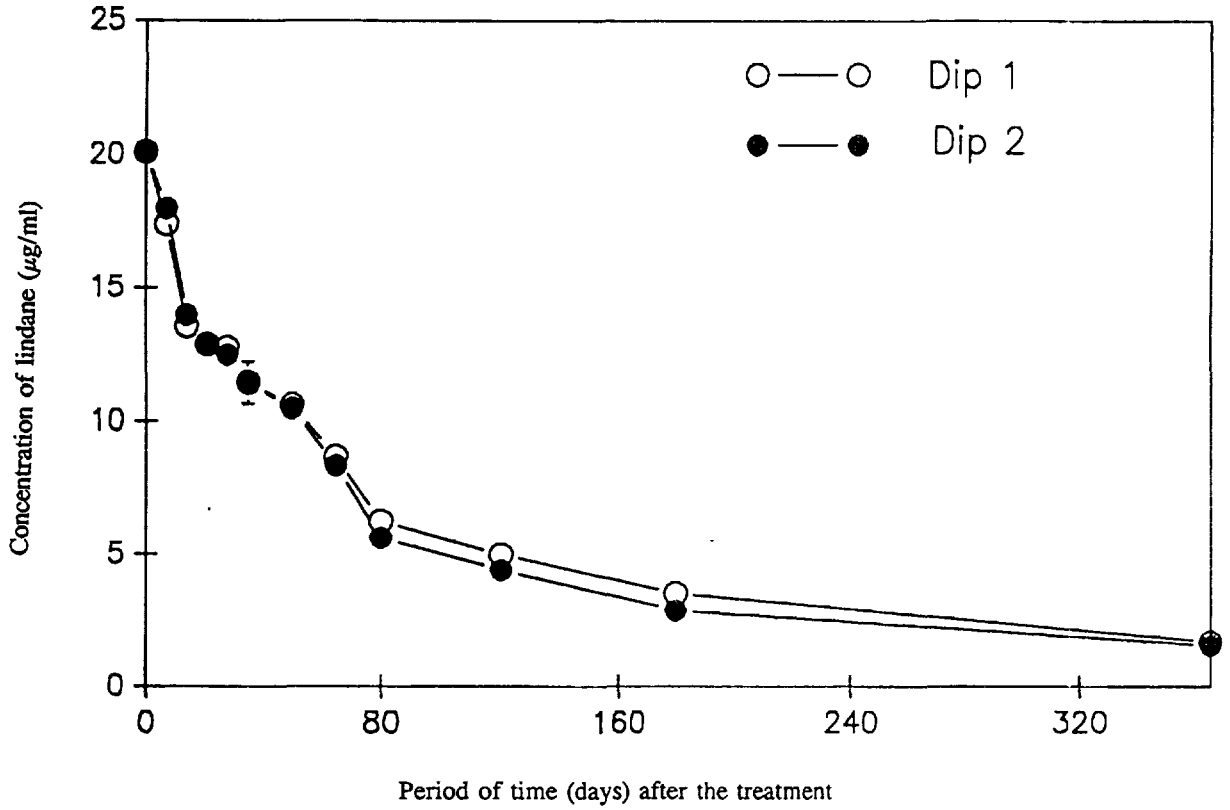

Figure 1. Dissipation of lindane in model dips

TABLE I. CONCENTRATION OF LINDANE IN MODEL CATTLE DIPS TREATED WITH ^{14}C-LABELLED LINDANE.

Days after treatment	Concentration of lindane (µg[1]/ml)	
	Dip 1[2]	Dip 2[3]
0	20.1 ± 0.04	20.1 ± 0.07
7	17.4 ± 0.15	18.0 ± 0.16
14	13.6 ± 0.16	14.0 ± 0.12
21	12.9 ± 0.12	12.9 ± 0.12
28	12.8 ± 0.43	12.5 ± 0.39
35	11.4 ± 0.78	11.4 ± 0.41
50	10.6 ± 0.13	10.5 ± 0.12
65	8.7 ± 0.30	8.4
80	6.3	5.6
120	5.0	4.4 ± 0.01
180	3.5 ± 0.02	2.9 ± 0.01
365	1.6 ± 0.14	1.5 ±

[1] Concentration of lindane was calculated from the measured radioactivity of the suspension
[2] Dip 1 was the control; no soil or cowdung was added
[3] Soil and cowdung mixture (1+1) was addded to Dip 2 28 days after treatment with lindane

In the second experiment, the same type of cattle dip with 50 L water was treated with 2.90 MBq ^{14}C and 1 g lindane in 25 mL acetone. The cattle dip was recharged at regular intervals of one month with 290 kBq and 100 mg unlabelled lindane in 25 mL acetone. Equal proportions of cowdung and soil were added before sampling as in the first experiment.

In another experiment the effect of pH (pH 5,6,7, 8 and 9) on the stability of lindane was studied. Water (1 L) was treated in glass jars with 74 kBq labelled and 50 mg unlabelled lindane for each pH. Three replicates of 20 mL from each experiment were taken for analysis. The sampling periods are given in Table II.

The total radioactivity in water was determined by using dioxane based scintillation fluid. The extraction of the residues was carried out by benzene. The recovery in benzene was about 99.4 ± 0.03 %. An alternate method was also developed for extraction of lindane from water. Lindane was extracted by hexane in the presence of small amount of methanol. The recovery by this method was 99+ %. The extract was concentrated by flash evaporator. This reduced the recovery by about 3%. Lindane and its metabolites were analyzed by Shimadzu model LC-4A HPLC using Zorbax ODS C_{18} RP column (25 cm length and 4.1 mm i.d.). Methanol at the rate of 0.5 ml per minute was used for elution. The absorbance was recorded at 225 nm. Four main peaks were obtained , three of which were identified by comparing their retention times with those of the standards as lindane, 1,2,4 -trichlorobenzene, 1,3,5-trichlorobenzene and unknown. The retention times of these peaks for lindane, 1,2,4-trichlorobenzene, 1,3,5-trichlorobenzene and an unidentified peak were 8.3±0.8, 10.0±0.65, 10.75± 0.73 and 10.5 ± 0.43 minutes, respectively. The fractions were collected separately and radioactivity estimated. The quantitative estimation of lindane and its metabolites was also carried out by determining the peak areas and using appropriate standard curves.

3. RESULTS AND DISCUSSION

3.1. First experiment

Each cattle dip containing 200 L water was treated with 2.46 MBq ^{14}C and 4 g unlabelled lindane. At zero time 744 dpm/mL were detected which declined to 132 dpm/mL 180 d after the treatment in the control dip. The total residues of lindane in terms of µg/mL are given in Table I. The lindane declined from about 20 µg/mL to 1.6 µg/mL within 365 d of treatment. Its percent dissipation is presented in Figure 1. It was seen that the dissipation of lindane was biphasic. The first phase was of rapid dissipation (0-14 d) followed by a slow phase (14 -365 d). The half life of lindane in the first phase was 25 d, however, the half life for the second phase was about 117 d. The overall half life of lindane was 101 d in model cattle dip. The half life of lindane at the same site in the soil was found to be between 124- 146 d [3] . The half life in water is slightly lower than in soil. When the extracts of lindane residues from the first dip (control) and second dip (experimental) were analysed by HPLC, only three peaks were found to contain radioactivity in the first experiment. The retention times of these peaks were identical with those of lindane, 1,2,4-trichlorobenzene and unknown. Of the three peaks obtained one could not be identified as yet. The concentration of lindane and its metabolites for the first experiment are shown in Table II. Lindane accounted for the bulk of the residues. However, 21d after treatment, 1,2,4-trichlorobenzene was detected and subsequently the unknown metabolite. Their concentrations increased gradually with time, reaching the maximum about 65 to 80 d after treatment. The proportions of lindane to its metabolites 1,2,4-trichlorobenzene and the unknown compound in dip 1 were 78.05 : 16.68 : 5.25 respectively 50 days after

TABLE II. CONCENTRATION OF LINDANE AND METABOLITES IN DIPS TREATED WITH ^{14}C-LABELLED LINDANE.

Days after Trt.	Concentration of lindane and metabolites (μg^1/ml)					
	Dip 1			Dip 2		
	Lind.[2]	124-TB[3]	Un-know[4]	Lind.	124-TB	Un-know
0	19.8	-	-	20.1	-	-
7	17.2	-	-	17.9	-	-
14	13.5	-	-	13.4	-	-
21	12.3	0.6	-	12.3	0.5	-
28	11.3	1.05	0.4	10.9	1.3	0.3
35	10.3	0.9	0.2	9.8	1.3	0.3
50	8.6	1.8	0.6	8.1	1.9	0.5
65	6.8	1.5	0.4	6.4	1.5	0.5
80	4.68	1.71	0.40	4.20	1.06	0.39
120	3.43	0.94	0.59	3.15	0.79	0.46
180	2.44	0.68	0.39	1.95	0.66	0.24

[1]Lindane and its metabolites were analysed by HPLC and quantitative estimation made from radioactivity.
[2]Lind. = Lindane
[3]TB = Trichlorobenzene
[4]Unknow = Unidentified metabolite

treatment. These values are 8.61, 1.84 and 0.58 µg/mL. The data for both dips is quite comparable.

3.2. Second experiment:

In the second experiment 16.31 µg/mL lindane (3638 dpm/mL) was present at zero time which was considered to be 100 %. This declined from 100 % at zero time to 11.69 % (1.9 µg/mL, 425 dpm/mL) after third recharge. The sampling schedule and amount of lindane in u/ml are given in Table III. After extraction of lindane residues by hexane these were concentrated and subjected to HPLC. Four peaks were found to contain radioactivity and were identified as lindane, 1,2,4- trichlorobenzene and 1,3,5- trichlorobenzene on the basis of their retention times. One peak before 1,3,5- trichlorobenzene could not be identified as

TABLE III. CONCENTRATION OF LINDANE IN CATTLE DIP AFTER RECHARGE AT REGULAR INTERVALS.

Days after treatment	Concentration of lindane	
	µg/mL	Percent of original
0	16.3 ± 0.1	100
1st recharge[1]	9.9 ± 0.2	60.7 ± 1.2
2nd recharge	7.9 ± 0.2	48.6 ± 1.0
3rd recharge	1.9 ± 0.1	11.7 ± 0.5

[1] Dip was recharged at monthly intervals with lindane equal to 10% of the original concentration.

TABLE IV. METABOLITES OF LINDANE IN DIP TREATED WITH ^{14}C -LABELLED LINDANE AND RECHARGED AT MONTHLY INTERVALS.

Sampling time	Concentration (µg/mL)[1]			
	Lindane	124-TB[2]	135-TB[3]	Unknown[4]
0 Days	16.5	-	-	-
After 1st recharge	7.5	2.1	0.2	0.1
After 2nd recharge	5.1	1.6	0.6	0.2

[1] Lindane and metabolites were identified by cochromatography on HPLC and quantitated by estimation of radioactivity on liquid scintillation counter.
[2] 124-TB = 1,2,4-Trichlorobenzene metabolite
[3] 134-TB = 1,3,4-Trichlorobenzene metabolite
[4] Unknown = One metabolite remained unidentified

TABLE V. EFFECT OF pH ON THE DISSIPATION OF LINDANE IN JARS.

Days after treatment	Concentration (μg/mL) of lindane in jars at different pH				
	pH 5	pH 6	pH 7	pH 8	pH 9
0	58.1±5.0	61.4±0.5	55.5±2.2	63.7±0.4	60.6±3.3
7	39.1±0.6	50.9±0.4	39.7±07	37.6±1.3	40.3±0.3
14	22.6±0.5	24.7±0.3	23.6±0.6	23.3±0.2	19.1±0.5
21	18.4	24.9±1.1	16.5±0.6	16.2±0.5	20.9±0.2
28	14.2	16.4	14.4±0.6	14.0±0.1	14.5±0.3
35	4.6±0.5	6.1±0.2	4.7±0.2	5.0±0.2	5.4±0.2

yet. The amount of lindane and its metabolites are given in Table IV. At zero time only lindane was present accounting for 100 % of the residues and its concentration was 16.45 μg/mL which then declined to about 5.10 μg/mL (68.08 %) after second recharge. 1,2,4-trichlorobenzene was found to be the main metabolite which accounted for about 21 % after 1st and 2nd recharge. The significant amount of 1,2,4 - trichlorobenzene may be due to regular recharge of cattle dip by 10 % fresh lindane which may accelerate the formation of this metabolite.

3.3. Effect of pH on persistence of lindane:

In the third experiment, effect of pH on the stability of lindane was studied and data are presented in Tables V. The amount of lindane was 58.14 μg at zero time (100 %) and this declined to 4.6 μg (7.9 %) in 44 d in water at the pH 5. The disappearance of lindane was slightly higher at pH 8 and was slightly less at pH 6.

ACKNOWLEDGEMENT

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A STUDY OF THE STABILITY OF COUMAPHOS, ^{14}C-LABELLED LINDANE AND ^{14}C-LABELLED CHLORPYRIFOS IN MODEL CATTLE DIP

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Abstract

The study was undertaken to evaluate the stability of coumaphos, ^{14}C-labelled lindane and chlorpyrifos insecticides/acaricides in a simulated cattle dipping vat. During the period of the study the pH of the suspension in vat increased due to addition of soil from the vacinity of cattle barn as well as due to standing in cement-concrete of the vat. The residual amounts of lindane and chlorpyrifos were estimated by counting the corresponding radioactivity in the suspension. Lindane dissipated rapidly and its half-life during the first phase was 4 d only. After standing for 3 weeks lindane residues were equivalent to 3.5% of the original amount in the suspension and 9.6% in the supernatent. Similarly, 97% of the original amount of lindane had dissipated in the soil sediment at the end of 3 weeks, indicating that dissipation of lindane was primarily due to volatilization. Chlorpyrifos was more stable under the experimental conditions and its half life was calculated to be 22 d during the first phase of dissipation. After 3 weeks the concentration of chlorpyriphos in the suspension remained almost unchanged. However, in the supernatant its concentration increased to 115% of the initial amount. The concentration of soil-bound residues of chlopyrofos remained less than 5% of the total amount applied through out the period of study.

The initial concentration of coumaphos in the suspension was 200 mg/L. During the period of study the residual concentration of coumaphos in the vats was measured by high performance liquid chromatography (HPLC) and the metabolites were identified by thin layer chromatography (TLC). The stability of coumaphos was found to be related to the pH of the suspension. In the first phase of dissipation the half life of coumaphos was 99 d. However, it was extended to 114 d on addition of superphosphate fertilizer. The addition of sodium citrate did not have any affect on the stability of coumaphos. Three metabolites of coumaphos were identified in the control vats as well as those treated with superphosphate or sodium citrate. These were chlorferon, potasan and an unidentified metabolite. The concentration of chlorferon decreased in the control vat as well as those treated with superphosphate or citrate. Potasan was dectectable during the first 30 - 90 d but not later.

1. INTRODUCTION

Ectoparasites such as ticks and mites are directly pathogenic to the host animal and are also involved in the transmission of many pathogenic organisms. Acaricides are chemicals which are used to control their infestation in livestock. The gamma-isomer of hexachlorocyclohexane (HCH), commonly known as lindane, is one of the most effective acaricides and is commonly used for the control of ticks in many countries. Similarly, among the organophosphorus compounds chlorpyrifos and coumaphos are highly effective against ticks in low concetrations.

In practice the acaricides are added to animal dipping vats and the animals are made to pass through the vats. Spraying and body washing of animal with acaricides are also practiced in many countries. But use of acaricide in animal dipping vats provide more thorough coverage and the same suspension is used repeatedly for the treatment of several animals. Therefore, this method of treatment is ultimately less costly in herd treatment. In addition, the use of dips allows better disposal of acaricidal residues. For efficient and cost effective ectoparasite control a minimum effective concentration and stability of acaricide in livestock dip are important. Therefore, regular monitoring and introduction of procedures to improve stability in the dip are essential.

The objective of the present study was to evaluate the stability of ^{14}C-labelled lindane, ^{14}C-labelled chlorpyrifos and coumaphos in simulated animal dipping vats under current practices and to develop procedures to improve their stability.

2. EXPERIMENTAL PROCEDURES

Simulated model vats were constructed with cement-concrete, each to hold 50 L water. These were placed under tamparature, light and air flow conditions simulating field conditions. Soil was collected form the vicinity of cattle barn and a portion equivalent to 1% of the weight of water content was added to each vat every month. A mixture of ^{14}C-labelled and unlabelled lindane (4 g) was dissolved in 50 ml acetone and added to 40 L water in each vat to give a concentration of 100 mg/L. Similarly, vats were treated with a mixture of ^{14}C-labelled and unlabelled chlorpyrifos. The level of water was maintained in each vat to a constant level through the period of study. Following the addition of acaricides in the model vat the solution was well mixed. The dissipation of the insecticides was monitored over a period of 9 weeks and the by analyzing samples taken at intervals shown in Table I. Triplicate samples of 0.5 mL were collected from each vat at each sampling time and radioactivity was estimated by counting in a liquid scintillation spectrometer. For analysis of residues in the sediment, the suspension containing the sediment was stirred well for several minutes and samples taken for analysis. A 10 mL aliquot was entrifuged at 3000 rpm for 10 minutes and then 0.5 mL of the supernatant was taken for measurement of radioactivity. Soil from each vat was collected by filtering well mixed suspension. One g soil sample was extracted for 4 h with methanol in Soxhlet apparatus and radioactivity in the methanol solution was estimated. Unextractable radioactivity remaining in the soil was estimated by combustion of the soil samples in a biological material oxidizer, absorption of the resulting $^{14}CO_2$ in liquid scintillation cocktail and analysis by liquid scintillation spectrometer.

In order to study the affect of additives on the stability of coumaphos, Asuntol powder (Bayer Co., Germany) containing 50% (w/w) coumaphos was suspended in the vat water to a concentration of 200 mg/L. To one vat 1% superphosphate fertilizer (containing a mixture of calcium sulphate and calcium acid phosphate) was added. To the second vat sodium citrate was added. The third vat served as the control. The temperature of the water during the experimental period varied from 20°C to 28°C. The change in the pH and residues of coumaphos in each vat were measured at each sampling time. A 100 mL sample of the suspension from each vat was extracted with ethyl acetate and the extract analysed by HPLC. The HPLC was equipped with a UV detector and a C-18 reverse phase column. The wavelength of the detector was adjusted at 254 nm. Acetonitrate+water (65+35, v/v) was used as mobile phase. The extract was also analysed by TLC. The TLC plates were developed in benzene+ethylacetate (9+1, v/v) solvent mixture. Asuntol and analytical standards were used for characterization/identification of the spots.

3. RESULTS AND DISCUSSION

Table I shows data on the change in the pH and the dissipation of lindane in the vat suspension and in the supernatant water. The pH on 0 day, before the addition of the soil, was 6.4. However, it increased steadily with time and reached a maximum of 9.5 after 7 weeks. The initial concentration of lindane in the suspension was 77.7 mg/L, which was below the calculated concentration of 100 ppm. The concentration in the supernatent was 35.5 mg/L. The dissipation seems to be biphasic as shown in Fig 1, with much faster loss of lindane during the first phase and somewhat slower dissipation in the second phase. The half-life in

the first phase was calculated to be 4 d. The half life of lindane has been reported to be 191 d at pH 7 and 11 h at pH 9 [1].

Data related to the dissipation of chlorpyrifos in model vats are shown in Table II. The initial concentration of chlorpyrifos in the suspension was 87.4 mg/L, but it steadily decreased

Table I. Dissipation of ^{14}C-labelled lindane in a model dip

Sampling period	pH	Concentration of lindane (mg/L)	
		In suspension	In supernatent
0 day	6.5[1]	77.7	35.5
2 days	8.4	55.5	22.0
1 week	8.7	31.9	13.9
3 weeks	9.0	2.7	3.4
5 weeks	9.0	1.1	1.4
7 weeks	9.5	0.6	0.7
9 weeks	9.3	0.3	0.4

[1] pH measured prior to the addition of the soil to the vat

Table II. Dissipation of ^{14}C-labelled chlorpyrifos in a model dip

Period of sampling	pH	Concentration of chlorpyrifos (mg/L)	
		In suspension	In supernatent
0 day	6.5[1]	87.4	8.5
2 days	8.5	76.4	7.8
1 week	8.7	44.3	8.1
3 weeks	9.0	42.4	8.6
5 weeks	9.3	29.9	9.9
7 weeks	9.1	30.0	9.4
9 weeks	9.1	32.2	9.8

[1] pH measured prior to the addition of the soil to the vat.

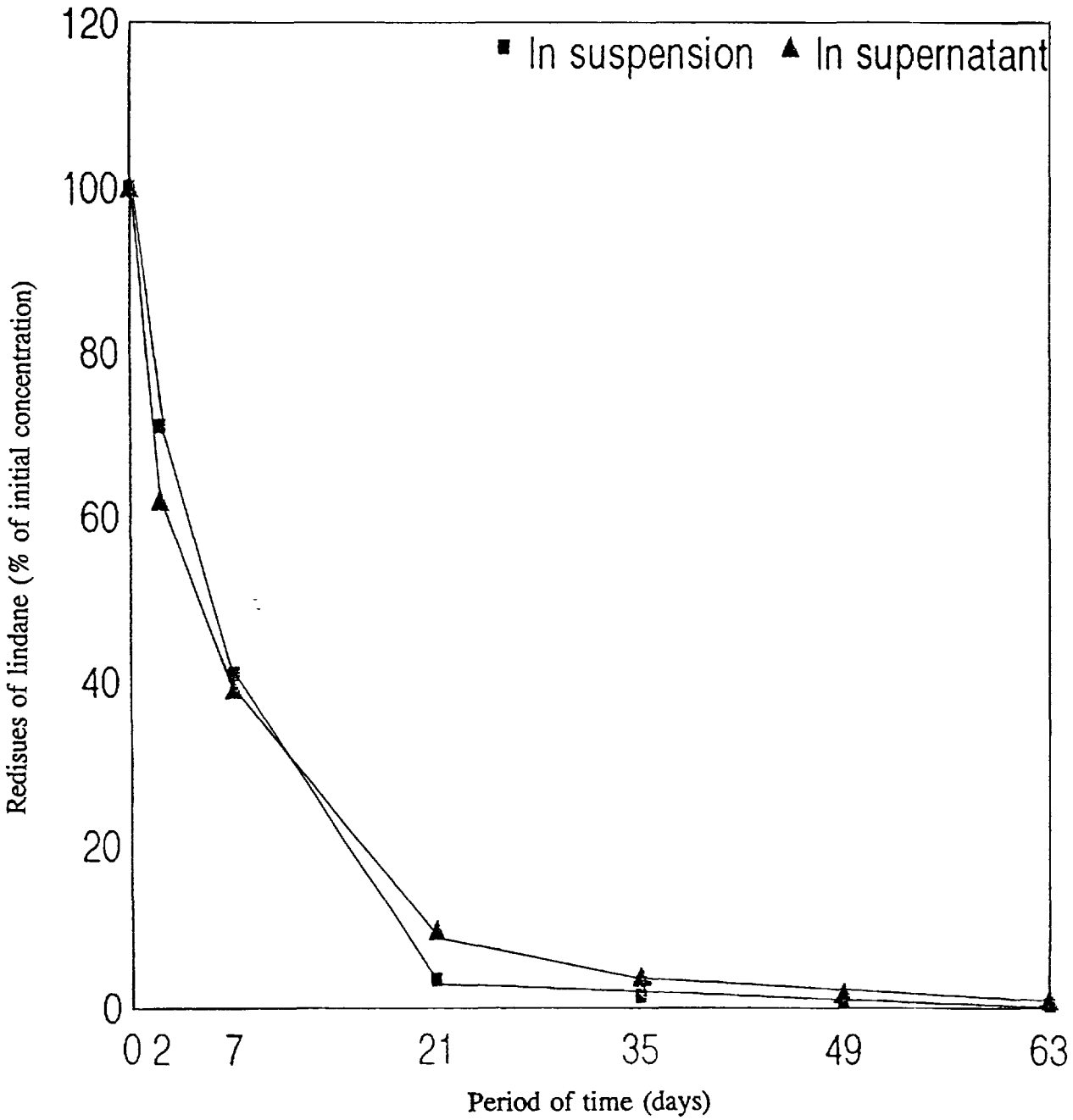

Fig. 1. Dissipation of lindane in a model dip.

to 32.2 mg/L after 9 weeks. On the other hand, the concentration of chlorpyrifos in the supernatent somewhat increased with time. This is because the concentration of chlorpyrifos of 100 mg/litre used in the suspension was much higher than its reported[1] maximum solubility of 1.4 mg/litre in water. Fig 2 shows a plot of the decline of chlorpyrifos in the suspension over the 9 week period. The dissipation of chlorpyrifos was slower than that of lindane. The half-life of chlorpyrifos was calculated to be 22 d. The initial pH of the suspension, before the addition of the soil, was 6.5. But later it increased, and reached a maximum of 9.1 after 9 weeks. This indicate a relationship between change of pH and the dissipation of chlorpyrifos. Chlorpyrifos undergoes hydrolysis and the rate of hydrolysis increases with pH. The half life of the insecticide in water has been reported to vary between 1.5 d at pH 8 (25°C) to 100 d at pH 7 (15°)[1].

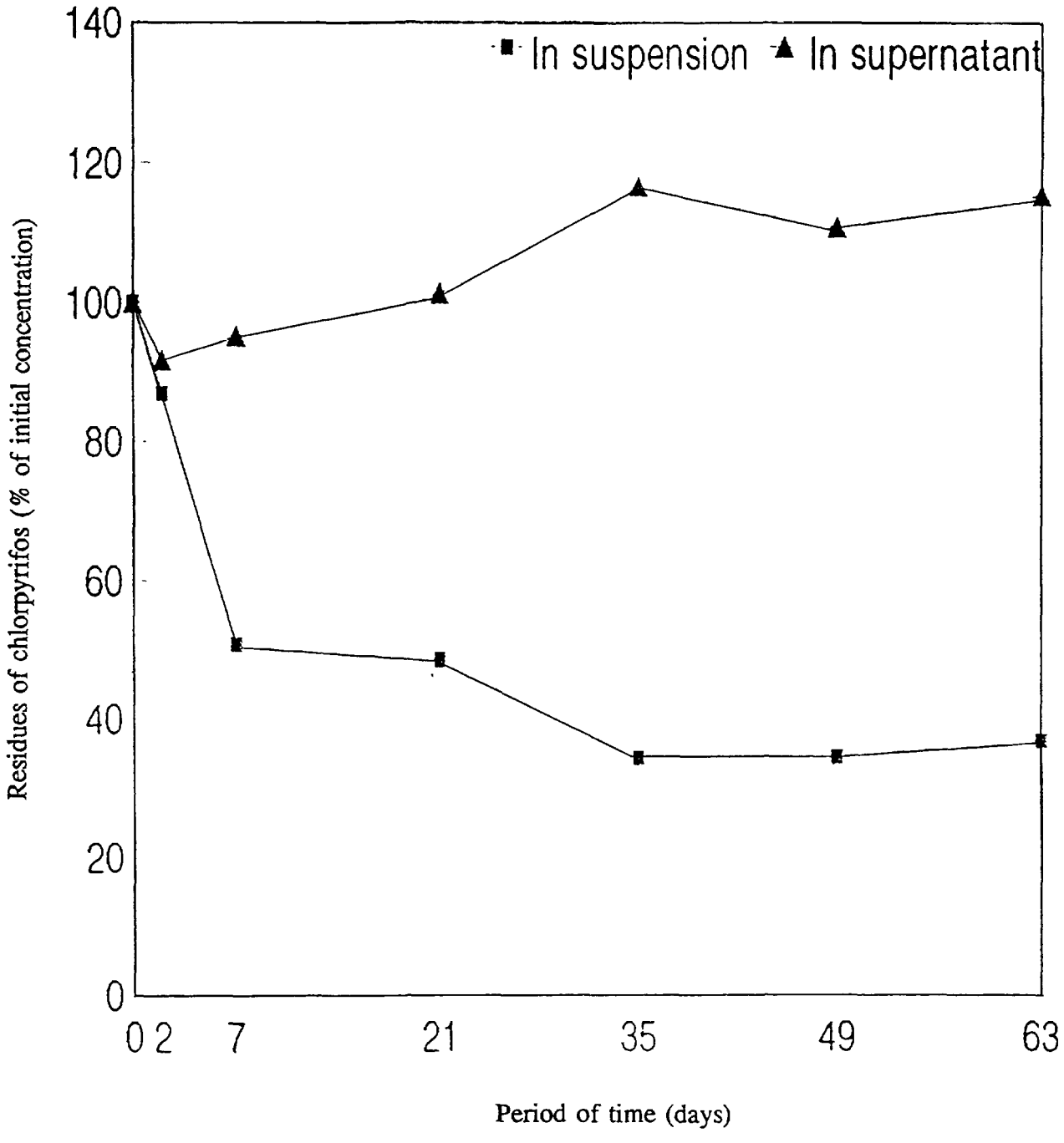

Fig. 2. Dissipation of chlorpyrifos in a model dip.

Methanol extractable and unextractable (bound) residues of lindane in soil/sediment taken from the model vat are shown in Table III. Analysis of the samples taken soon after the application of lindane to the vat showed total lindane residues of 75.3 µg/g which included 64.1 µg/g extractable and 11.2 µg/g (14.9%) unextractable. After 3 weeks the total residues in the soil had decreased to 5.0 µg/g which included 50% extractable and the rest unextractable. At the end of 9 weeks total residues of lindane in the soil/sediment were only 1.2 µg/g, indicating rapid dissipation from soil.

Table IV shows data on methanol extractable and non-extractable residues of chlorpyrifos in the soil/sediment. The concentration of chlorpyrifos in the soil/sediment soon after the

Table III. Extractable and unextractable residues of lindane in the soil/sediment of model dip

	Residues of lindane in soil (μg/g)		
		Unextractable	
Period of sampling	Extractable	quantity (μg/g)	% of total residues in soil
0 day	64.1	11.2	14.9
3 weeks	2.5	2.5	50.0
6 weeks	1.0	1.5	60.0
9 weeks	0.5	0.7	58.3

Table IV. Extractable and unextractable residues of chlorpyrifos in soil/sediment of model dip

	Residues of chlorpyrifos in soil (μg/g)		
		Unextractable	
Period of sampling	Extractable	Quantity (μg/g)	% of total residues in soil
0 day	323.0	15.4	4.5
3 weeks	191.6	7.8	3.9
6 weeks	84.8	3.8	4.3
9 weeks	79.9	2.4	3.0

application of the insecticide to the vat was 338.4 μg/g which included 323.0 μg/g extractable and 15.4 μg/g unextractable. The total residues in the soil decreased with time and after 9 weeks only 82.3 μg/g chlorpyrifos was present. Most of the residues were extractable and the proportion of soil/sediment bound residues did not excede the initial concentration of 4.5 μg/g.

Data related to the degradation of coumaphos in model vats are shown in Table V. The initial pH of the water before adding to the vats was neutral. However, after it was added to the cement vats the pH increased to 8.5. The addition of superphosphate decreased the pH to 6.4. The pH in this vat slowly increased to 7.0 in 60 days and 8.2 after 180 d. The pH of the vat treated with sodium citrate, on the other hand, was 8.9 and changed very little through

Table V. The effect of additives on pH of the suspension and the dissipation of coumaphos in a model dip.

Period of time	Control		With super phosphate		With sodium citrate	
	pH	Residues of coumaphos (mg/L)	pH	Residues of coumaphos (mg/L)	pH	Residues of coumaphos (mg/L)
0	8.5	204.2 ± 2.6	6.4	209.5 ± 18.3	8.9	189.00 ± 7.6
15	8.5	197.7	6.5	217.2 ± 10.8	8.8	183.9 ± 7.1
30	8.6	184.1 ± 9.8	6.8	195.8 ± 0.2	8.8	183.6 ± 16.9
60	8.5	126.5 ± 0.7	7.0	133.3 ± 2.6	8.9	115.0 ± 1.3
90	8.5	101.0 ± 1.0	8.0	117.1 ± 2.8	9.1	89.7 ± 33.2
120	8.5	88.6 ± 1.9	8.3	101.2 ± 8.4	9.0	81.0 ± 19.7
150	8.4	93.0 ± 4.2	8.4	94.1 ± 1.4	9.0	89.9 ± 7.2
180	8.6	92.8 ± 4.7	8.2	95.9 ± 3.9	9.2	88.7 ± 5.5

Table VI. Concentration of chlorferon metabolite of coumaphos in model dips at different sampling times.

Period of time (days)	Control model dip	With super phosphate	With sodium citrate
	Chlorferon concentration (mg/L)	Chlorferon concentration (mg/L)	Chlorferon concentration (mg/L)
0	16.5 ± 2.6	14.0 ± 1.8	13.4 ± 0.2
15	14.2 ± 0.5	16.1 ± 0.6	14.2 ± 0.5
30	15.3 ± 0.4	16.9 ± 2.2	18.2 ± 0.8
60	14.2 ± 1.0	10.9 ± 0.5	12.8 ± 0.6
90	8.8	8.5	11.9 ± 1.0
120	8.8	9.2 ± 0.5	9.8
150	8.7 ± 0.1	8.1 ± 0.4	8.9 ± 0.2
180	7.1 ± 2.1	8.2 ± 0.5	8.1 ± 0.8

Table VII. Concentration of potasan metabolite of coumaphos in model dips at different sampling times.

Period of time (days)	Control model dip	With super phosphate	With sodium citrate
	Potasan concentration (mg/L)	Potasan concentration (mg/L)	Potasan concentration (mg/L)
0	4.1	-	3.8 ± 0.2
15	3.1 ± 0.3	3.9	3.5 ± 0.3
30	3.2 ± 0.2	3.8 ± 0.1	4.2 ± 0.2
60	-	-	2.7
90	-	2.2	-
120	-	-	-
150	-	-	-
180	-	-	-

Table VIII. Rf value of coumaphos and metabolites on the TLC plates developed in different proportions of the solvent mixture

Compound	Rf (solvent mixture A[1])	Rf (solvent mixture B[2])
1. Individual standards[3]		
Chloroferon	0.44	0.63
Potasan	0.74	0.80
Coumaphos	-	0.89
2. Mixed standards[4]		
chloroferon	0.44	0.65
potasan	0.78	0.83
coumaphos	0.90	0.87
unidentified metabolite	nd	0.91
3. In sample extract[5]		
chloroferon	0.44	0.62
coumaphos	0.88	0.87
unidentified metabolite	0.96	0.93

[1]Solvent mixture A was benzene+ethylacetate in 90+10 (v/v) ratio.
[2]Solvent mixture B was benzene+ethylacatate in 70+30 (v/v) ratio.
[3]Rf of standards of compounds applied to TLC plate individually.
[4]Rf of compounds applied to TLC plate as a mixture of standards.
[5]Rf of compounds when the extract was applied to TLC plate.

the period of experiment. The initial measured concentration of coumaphos was betwwen 189 ±7.6 and 209 ±18.3 mg/L. However, it steadily decreased in all three vats with time. The data indicate that during the first 120 d the concentration of coumaphos in the vat treated with superphosphate remained somewhat higher than the other two vats, evidently due to lower pH. However, the final concentration in the three vats was roughly the same after 180 days. The addition of superphosphate fertilizer stabilized the acaricide for a month or so but thereafter its affect diminished. Obviously, in order to repeated addition of superphosphate to the vat would be stabilize the acaricide for a longer period of time. The addition of sodium citrate to the vat did not have any significant affect on the stability of the acaricide. From the data in Table V the half life of coumaphos was calculated to be 99 d in the control vat and the addition of superphosphate prolonged it to 114 d. The half life of the acaricide in the vat treated with sodium citrate was calculated to be 98 d.

HPLC and TLC analysis of the radioactivity extracted from the vat suspension showed the presence of three metabolites in addition to coumaphos in all three vats. The meatbolites included chlorferon, potasan and an unidentified metabolite. The concentrations of the identified metabolites are shown in Tables VI and VII. The Rf values of coumaphos and metabolites on the TLC plates developed in two solvent mixtures are shown in Table VIII. The initial presence of chlorferon in the control vat indicates that Asuntol had this metabolite already present. However, its concentration steadily decreased with time. The concentration of this metabolite in the vats treated with superphosphate fertilizer of sodium citrate slightly increased during the first month but after that it started to decrease. The concentration of potasan remained low in all three vats.

It can be concluded from this study that lindane dissipates from the model animal dipping vats quite rapidly, mostly due to volatilization, with a half life of only 4 days. Chlorpyrifos, on the other hand, is somewhat more stable and has a half life of 22 days. The degradation of coumaphos can be slowed down by the addition of superphosphate fertilizer but is not affected by the addition of sodium citrate. However, in order to stabilize coumaphos repeated addition of superphosphate may be necessary.

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DEVELOPMENT OF PROCEDURES TO STABILIZE CHLORFENVINPHOS IN MODEL CATTLE DIPS

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Abstract

The environmental fate and dissipation of chlorfenvinphos acaricide was studied in water and sediment in model cattle dips with recharge, without recharge and with added stabilizers. Chlorfenvinphos concentration decreased with time in all of them, and the monthly recharge at 10% of the initial concentration was inefficient to maintain a concentration that would be effective for tick control. However, the loss of pesticide in the model dip with added phosphate buffer as stabilizer was the least. Volatilization was the principal factor that influenced the dissipation of the pesticide. The sediment bound residues increased with time. Mineralization of ^{14}C-chlorfenvinphos due to microbial activity showed that the $^{14}CO_2$ production increased with time in biometers flasks with different amounts of sediment. 2,4-Dichloroacetophenone, and 2,4-dichlorobenzaldehyde were identified as degradation products. Isomerization of chlorfenviphos from isomer Z to E was influenced by sunlight. Chlorfenvinphos was stable in aqueous solution for 14 days under pH 4 to 9. Leaching tests demonstrated that the pesticide was not a potential pollutant of ground water.

1. INTRODUCTION

The Ecuadorean cattle ranchers have serious problems due to cattle tick infestation. The tick acts as a vector in disease transmission and can cause serious economic losses. At the present time the tick control is based on the use of acaricides by several methods such as the use of sprayers, dust bags and cattle dips. However, the most efficient and widely applied method in the country is the use of dips. It is very important to use pesticides at the right concentration in order to have an efficient control and to avoid the development of resistance. Moreover, it is important to know the principal routes of acaricides degradation in the cattle dips, which will help in improving their stability and in reducing the high cost associated with their rapid degradation. It is also important to dispose off used contents of cattle dips after their use in order to avoid their adverse environmental effects.

Under controlled conditions, the use of nuclear techniques is very useful to monitor the fate of radiolabelled pesticides and to identify their residues at trace levels. One of the commonly used pesticides in cattle dips is the organophosphate chlorfenvinphos. The pesticide is sold in Ecuador under the trade name of Steladon. The commercial formulation is composed of a mixture of the Z and E isomers in 90: 10 (w/w) ratio, respectively.

The objectives of this research were to evaluate the stability of chlorfenvinphos in model cattle dips, test procedures to improve their stability, determine the influence of the microorganisms on the degradation of the pesticide, study the leaching behaviour of the pesticide after disposing in the soil and study the stability of chlorfenvinphos exposed to sunlight.

2. MATERIALS AND METHODS

2.1. ^{14}C-labelled and unlabelled chlorfenvinphos, metabolites and formulation

^{14}C-Chlorfenvinphos, labelled in ethyl moiety, with specific activity of 1.21 MBq/mg was supplied by the International Atomic Energy Agency's Laboratory at Seibersdorf, Austria. The analytical standards of chlorfenvinphos (Z+E) and the individual isomers Z and E were kindly

donated by CIBA GEIGY Limited, Basilea, Switzerland, and Shell Research Limited, Sittingbourne, Kent, U.K. The standards of 2,4-dichloroacetophenone and 2,4-dichlorobenzaldehyde were obtained from Chem Service Inc., Tower Lane Chester, U.S.A. The formulation of chlorfenvinphos, which is locally used to control cattle ticks was purchased in Quito, Ecuador

2.2. Determination of the purity of ^{14}C-chlorfenvinphos.

The purity of the pesticide was determined on thin layer chromatography plates developed in two solvent systems: (1) toluene+ethyl acetate+ethanol (20+75+5, v/v/v) and (2) toluene+ethyl acetate (80+20, v/v). The purity of chlorfenvinphos was 75.2% in system 1 and in 72.8% in system 2.

2.3. Purification of ^{14}C-chlorfenvinphos.

Because the radiolabelled pesticide did not have the desired purity of 95+%, it was further purified by using a preparative silica-gel plate (Aldrich). An aliquot containing 18.5 MBq ^{14}C-chlorfenvinphos dissolved in 650 µL benzene was applied on the plate and the plate developed in solvent system 2. The zone which contained the pesticide was scrapped and extracted with 2 x 10 mL acetone and 10 mL methanol. The extract was mixed for 10 min., diluted to 30 ml with acetone, filtrated through a 20 µm filter disk and concentrated to 5 mL. After the purification, the purity of chlorfenvinphos was 96- 97 %.

2.4. Determination of chlorfenvinphos concentration in the commercial formulation

Chlorfenvinphos concentration in Esteladon 300 formulation was determined by GC/NPD in phosphorus mode. The operating conditions for the GC were as following: injector temperature 220 °C, detector temperature 280 °C, H_2 flow rate 46 ml/min, air 280 ml/min and N_2 20 ml/min. The GC was equipped with a DB1 megabore column (0.53 mm x 30 m x 1.5 µm). The following temperature programme was used: initial temperature 80 °C, hold time 1 min, ramp rate 30 °C/min, final temperature 250 °C for 3 min. The formulation was found to contain 308 g per liter of active ingredient.

2.5. Determination of moisture content and organic matter in the sediment.

For the determination of moisture content of the sediment used in the model dips and biometer flasks, the sediment was dried in an oven at 110 °C for 24 hours. The total organic matter was determined using a colorimetric method in the samples previously digested and oxidized with sulfuric acid and potassium dichromate.

2.6. Assembly of model dips.

In order to simulate the current field practices, two plastic tanks, each with 60 litre capacity, were built, fitted inside wooden boxes and placed outdoors. Roofs were constructed above the tanks to protect them from the rain. The tanks were surrounded by wood shavings for insulation and the shavings were covered with sediment collected from the viscinity of a field dip. The model closely simulated a field dip. The tank was filled with 40 L of water. A stock solution of ^{14}C-labelled chlorfenvinphos was prepared by transferring 4.5 mL ^{14}C-chlorfenvinphos solution (containing 9.42 mg chlorfenvinphos) and 125 mL of Esteladon formulation (containing 38.8 g AI) into a 200 mL volumetric flask. The volume was braught to the mark with acetone. The total activity of the solution was 11.47 MBq. To each tank 65

mL stock solution was added to give a final concentration of 250 µg/mL and 3.7 MBq radioactivity in 50 L. To each tank 50 g (oven dried wight) sediment collected from the field was also added. The contents of the dips were well mixed and the initial concentration determined by taking 3 x 1 mL samples from each tank and counting the radioactivity in a Packard liquid scintillation counter (LSC). All samples were quench corrected by means of external and internal standards methods.

2.6.1. Model dips with added copper sulphate, superphosphate fertilizer or both:

A stock solution containing chlorfenvinphos formulation (18.75 g AI) was spiked with 100 mL acetone solution of ^{14}C-chlorfenvinphos (4.56 mg). Three model dips containing water were treated, each with 27 mL of the stock solution. To the first dip 100 mg copper sulphate and 20 g sediment (oven dreid weight), to the second dip 25 g superphosphate fertilizer (acidic buffer) and 20 g sediment and to the third dip superphosphate fertilizer and copper sulphate as well as sediment added and the volume in each tank made up to 20 L. Thus, each dip contained 250 µg/mL chlorfenvinphos and 1.48 MBq radioactivity.

2.7. Sampling of water and sediment and addition of sediment to the model dips

Samples from each dip were taken every 15 d. The liquid contained in each dip was homogenized and 3 x 1 mL sub-samples taken for direct radioassay by LSC. Samples (3 g) of sediment were taken from each dip every thirty days. After every fifteen days 50g sediment (oven-dry basis) was added to each dip to simulate passage of cattle through the dips.

2.8. Monthly recharge of the model dips.

To simulate the field conditions and to compare the differences between the dips with recharge and without recharge, one dip was recharged once every month with 10% of the initial pesticide concentration and radioactivity. In order to simulate the loss of water caused by the pass of cattle through the dip 5 L of suspension were removed from the dip and replaced with 5 L fresh water. To this 6 mL of the stock solution was added and the solution was mixed.

2.9. Analysis of the suspension

The suspension in each dip was analysed by taking 5 ml samples. The samples were homogenized and centrifuged for 10 min. The pH of the supernatent was determined and 2 x 1 mL sub-samples radioassayed. The suspension samples (4 mL) were extracted with ethyl acetate and the extract was dried on sodium sulfate. The recovery of extraction was 93%. The organic phase was analyzed by GC/FPD which was operated under the following conditions: injector temperature 230 °C, detector temperature 250 °C. The gas flow rates were: H_2 125 mL/min, air 250 mL/min and N_2 40 mL/min. The GC was equipped with a DB-1701 megabore column (0.53 mm x 30 m x 1.0 µm). The temperature was programmed as following: initial temperature 200 °C, hold time 3 min, ramp rate 20 °C/min, final temperature 240 °C for 1 min. A 2 mL aliquot of the organic phase were concentrated to near dryness using nitrogen, using isoctane as keeper. The concentrated solution was cleaned on a small silica gel column, which was eluted with a mixture of hexane+ethyl acetate (6+4, v/v). The cleaned material was analysed by GC/ECD for chlorfenvinphos metabolites. The operating conditions for GC/ECD were as following: injection port temperature 220 °C, detector temperature 310 °C. The flow rates were as following: 10 mL/min for N_2 as carrier and 30 mL/min for N_2 as make up. The GC was equipped with a megabore column RTX1 (0.53 mm x 30 m x 1.5 µm)

and operated under the following temperature programme: initial temperature 140 °C, hold time 1 min, ramp rate 5 °C/min, final temperature 200 °C for 1 min.

2.10. Treatment of sediment and determination of extractable and bound residues.

The sediment collected from each cattle dip was air dried for 24 h and homogenized. Three samples of 100 mg were combusted in a Harvey OX-600 Biological Oxidizer. Sediment samples were extracted in a Soxhlet apparatus with methanol for four hours. Bound residues were estimated by combusting triplicate samples in the oxidiser. The methanol extracts were concentrated and the extracts analysed by TLC. TLC spots for the metabolites were scrapped, extracted with ethyl acetate and analyzed by GC/ECD.

2.11. Study of the degradation of chlorfenvinphos from microbial activity.

Degradation of chlorfenvinphos due to microbial activity was studied in biometer flasks. A total of 18 biometer flasks were used. Six flasks (1X) were charged each with 75 mL field water, 1.72 g sediment and 250 µL stock solution of ^{14}C-chlorfenvinphos to obtain a final acaricide concentration of 250 µg/mL and radioactivity of 5.55 kBq. A second batch of 6 biometer flasks (5X) were charged with 75 mL water, 8.63 g sediment and 250 µL stock solution. Four additional flasks were charged, two each similar to 1X and 5X and foam plugs were inserted between the flask and the side arm to trap any volatile effluents. The plugs were extracted with 100 ml methanol and radioassayed. Finally, 2 biometer flasks were charged similar to 5X, autoclaved for 24 hours to prevent microbial activity and were used as control. The side arms of the flasks were charged with 10 mL monoethanol amine + methanol solution (20 %) to trap $^{14}CO_2$ produced as a result of the microbial metabolism. Every 8th day samples from the solution in the side arm was analysed for radioactivity and the solution was replaced with fresh amine solution. This procedure was followed for a 10 week period. After 4 and 10 weeks of incubation the sediment was extracted and bound and extractable residues were determined in 3 1X and 3 5X flasks. Water was extracted and analyzed by GC/FPD for chlorfenvinphos and by CG/ECD for metabolites. Samples of water were also acidified, extracted and analysed by TLC for diethyl phosphate residues. The TLC plates were developed in a mixture of isobutanol + isopropanol + ethanol + water (25+20+20+34, v/v/v/v) and sprayed with acid molybdate reagent (Hanes reagent).

2.12. Photoisomerization of chlorfenvinphos isomers.

In order to study the effect of sunlight on the isomerization of chlorfenvinphos (conversion from isomer Z to E) a test was carried out in which a 50 ng/µL (x 3) solution of chlorfenvinphos analytical standard was applied on glass plates which were exposed to sunlight for a period of 8 h. At the end of this period the glass plates were washed with 2 ml ethyl acetate and analysed by GC/FPD.

2.13. The effect of pH on the stability of chlorfenvinphos

Phosphate buffer solutions of pH 4, 5, 6, 7 and 9 were prepared using commercial buffers (Metrepak). Two 40 mL aliquots of each solution were transferred in to flasks. To each flask 1 ml stock solution containing 0.8 mg mixture of non-labelled and ^{14}C-chlorfenvinphos (55.5 kBq radioactivity) was added giving a final concentration of 20 µg/mL. The flasks were capped and the solutions homogenized for 14 d. Samples (1 mL) were taken at 0, 3, 7, 10 and 14 days and directly radioassayed. Also, samples were extracted with ethyl acetate and analyzed by TLC and GC/FPD in order to determine the effect of pH on the stability of chlorfenvinphos.

2.14. Chlorfenvinphos leaching assay

PVC pipes of 40 cm length and 4.5 cm diameter were inserted in the ground in the coastal region where the cattle dips are used. Soil around the columns was removed periodically in order to reduce compaction inside the columns. The soil had 27 to 40% clay, less than 20% sand and the following content of organic matter: 6.0% in the first section, 5.4% in the second and 3.6% in the third and fourth sections. The field capacity of the soil was 44%. A gauze pad was placed on the bottom of each PVC column. The columns were taken out of the ground, transferred to the laboratory, 230 mL water was added to each column and columns were left to drain freely for 48 hours in order to reach field capacity. A 1250 mL sample (x 3) of the final suspension was taken from the dips treated with ^{14}C-chlorfenvinphos and copper sulfate or superphosphate and passed through three columns for each type of suspension. It took about three days for the suspension to pass through each column. The leachate was collected and radioanalyzed. After two weeks, 102 mL distilled water was added to each column in order to simulate the natural rain. This amount of water represented a mean precipitation during the last ten years in the area. Four such simulated rains were made on each column weekly and the leachates were collected and analysed for pesticide content.

After water from the last simulated rain had drained the columns were cut at 5 cm intervals. The soil in each segment was mixed and weighed. Two samples (5 g) from each segment were used to determine the moisture content and three samples (1 g) were used to determine the total ^{14}C by combustion in the biological oxidizer. The soil was extracted and extractable and bound residues were determined. Samples of 35 g from each section were extracted in a Soxhlet apparatus with 300 mL methanol for 8 hours. Bound residues were determined by combusting 1 g soil samples after Soxhlet extraction.

3. RESULTS AND DISCUSION

3.1. Dissipation of chlorfenvinphos in model dips with and without recharge.

The initial concentration of chlorfenvinphos in the suspension was 250 µg/mL, but it decreased with time in all treatments. As shown in Fig. 1 the decrease was more steady when there was no recharge and no stabilizers were added. The dissipation was biphasic. The half life for first phase was calculated to be 5 weeks and for the second phase 18 weeks. The overall half life was 6.2 weeks. As shown in Fig. 2, with monthly recharge also the initial dissipation was rapid. The overall half life in this case was 8 weeks. Thus, although the recharge extended the half life of the acaricide somewhat, it was not enough to maintain the concentration at an effective level. The pH of the solution was initially 7 and fluctuated between 6 and 7 through the period of study. Most of the residues in the sediment were extractable. .

3.2. Dissipation of chlorfenvinphos in model dips with added stabilizers

The dips treated with superphosphate, copper sulphate or both as stabilizer were monitored for a period of sixteen weeks. The concentration of pesticide in the suspension decreased to 36-39 % of the original concentrationas as shown in Figure 3, and there was no significant difference between the three treatments. After standing for 16 weeks the concentration of chlorfenvinphos was somewhat higher in the dips treated with the stabilizers (Fig. 3) than the dip without recharge and without stabilizers (Fig. 1). The half life of the acaricide was calculate to be 8.7, 5.7 and 7.5 weeks in dips treated with superphosphate, copper sulphate and the mixture, respectively. Thus, it appears that the addition of superphosphate fertilizer

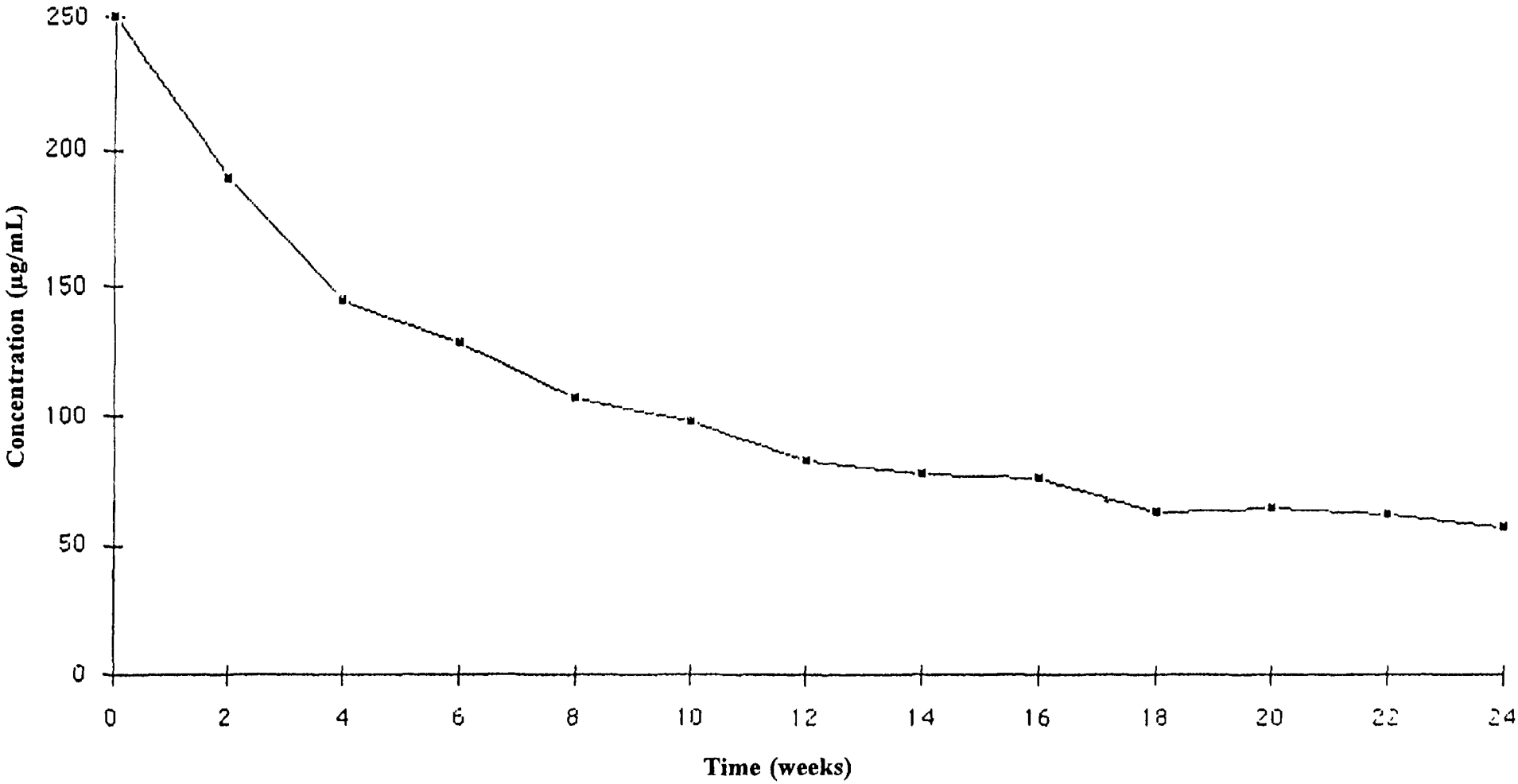


Fig. 1. Residual concentration of chlorfenvinphos in model dip without recharge measured at different periods of time.

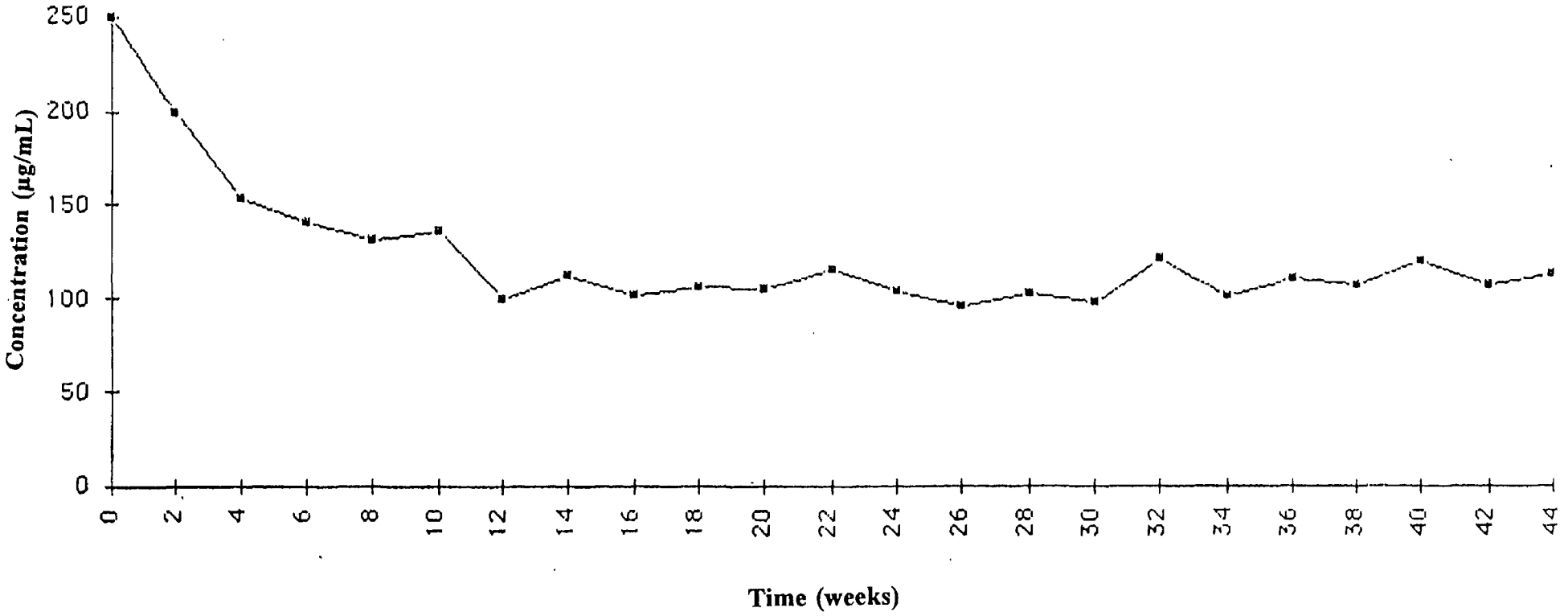


Fig 2. Residual concentration of chlorfenvinphos in model dip with monthly recharge measured at different periods of time.

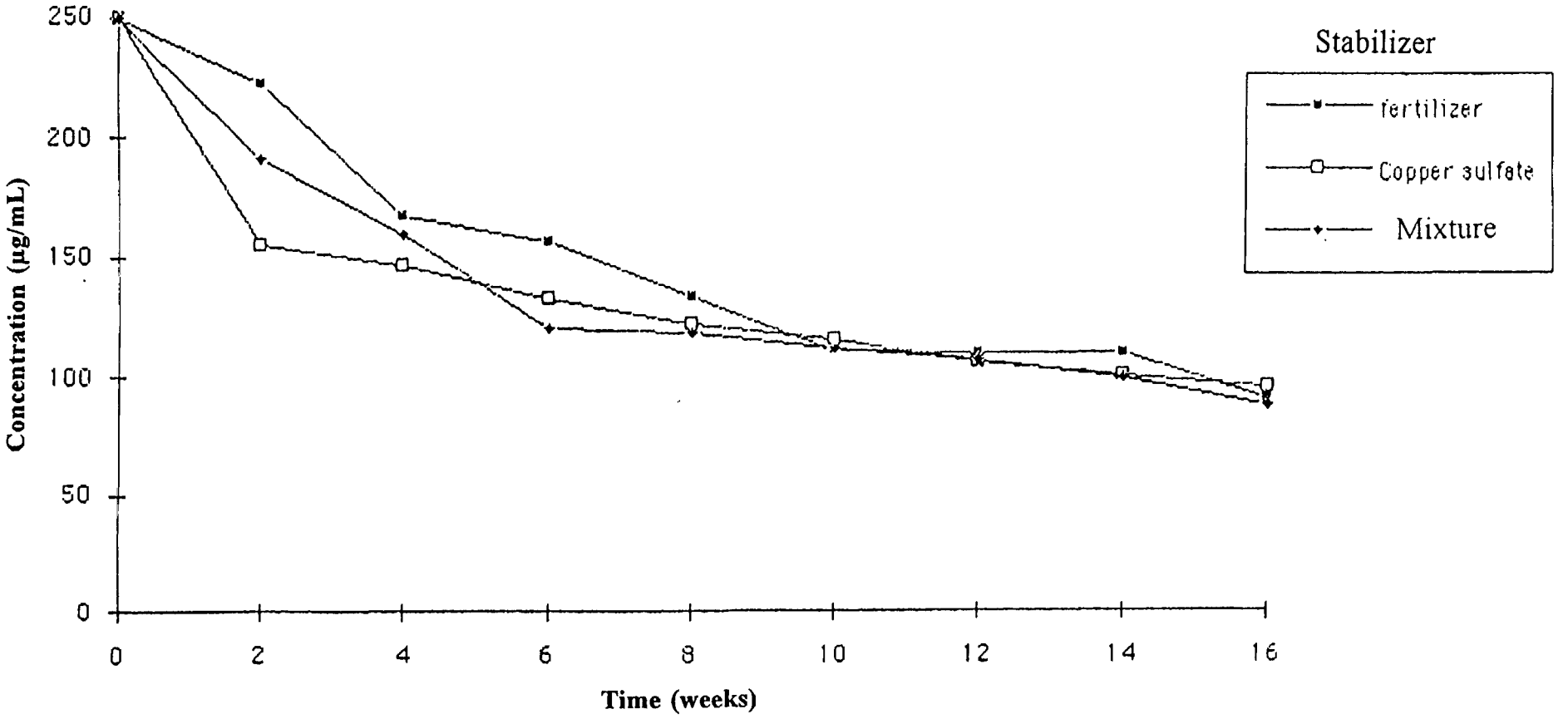


Fig 3. Residual concentration of chlorfenvinphos in model dips with added stabilizers measured at different periods of time.

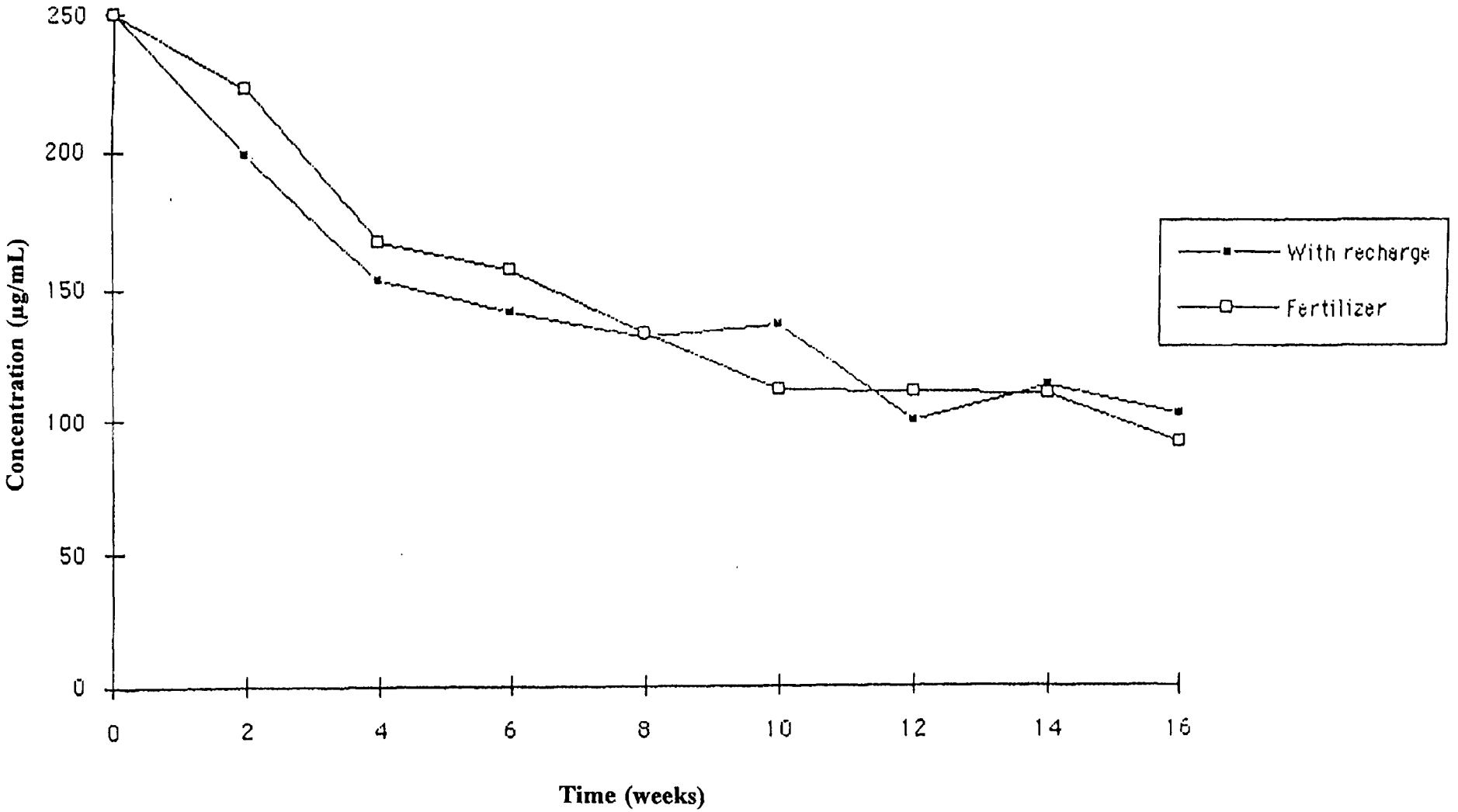


Fig. 4. Residual concentration of chlorfenvinphos in model dip with added superphosphate fertilizer and monthly recharge

had some effect on the stabilization of chlorfenvinphos. Fig. 4 shows a comparison between the dip with monthly recharge with the dip with superphosphate fertilizer, the stability of the two dips is quite similar. In the dips treated with superphosphate fertilizer or a mixture of the fertilizer and copper sulphate the pH fluctuated between 5.2 and 5.8, and in the dip treated with copper sulphate it ranged between 6.3 and 7.1. More than 99% of the residues in the sediment were extractable.

The results obtained by radiometric assay (Figs. 1-3) were confirmed when the samples were analysed by GC. Two metabolites, 2,4-dichloroacetophenone and 2,4-dichloro benzaldehyde were confirmed by GC analysis of the sediment extract. These metabolites were also identified in the dips treated with the stabilizers. The concentration of the metabolites after 16 weeks was as shown below:

Type of dip	2,4-dichlorobenzaldehyde ng/µL	2,4-dichloroacetophenone ng/µL
Buffer dip	0.01	0.02
Copper dip	ND	0.05
Mix dip	0.02	0.08

3.3. Dissipation of chlorfenvinphos in biometer flasks.

Experiments using biometer flasks were carried out to determine the mineralization rate of chlorfenvinphos. Different quantities of sediment containing organic matter content of 6.9% were used. Table I shows data on the evolution of $^{14}CO_2$ in the two sets of biometer flasks. The evolution of $^{14}CO_2$ increased with time. In the flasks containing 1X sediment cumulative radioactivity due to $^{14}CO_2$ was 2.4% of the initial amount of radioactivity in the suspension. In the flasks containing 5X the cumulative radioactivity after 10 weeks of incubation was 6.6%. Low background radioactivity was found in the flasks which were sterilized. This assured that the mineralization of the acaricide was caused by the microbial organisms present in the system. The initial low level evolution of the $^{14}CO_2$ can be explained due to the adaptation of the microbes to the substrate. Later on faster increment in the percentage of $^{14}CO_2$ formed is due to an exponential growth of the adapted microbial population.

Chlorfenvinphos concentration determined at the end of the test in biometer water samples by GC/FPD was 83.7 µg/mL and 56.3 µg/mL for the biometer flasks 1X and 5X respectively, the diference can be explained due to the higher quantity of sediment and microorganis responsables for the mineralization. The pH increased from 7.01 to 9.85 and probably it helped the mineralization of the pesticide. The bound residues in the sediment increased during the assay reaching 22.2 % and 21.2 % in the 1X and 5X biometers respectively. Those figures were higher than the bound residues founded in the dips, but its important to notice that the alcaline pH and the anaerobic medium present in the biometer flasks may contribute to the formation of bound residues. McMinn et.al. [1] studied chlorfenvinphos degradation under anaerobic conditions in soil and found an increase in the formation of bound residues after eight weeks of soil incubation. The organic matter content in the sediment used in the biometer flasks can contribute to the microorganisms growth and an increase in pesticide degradation can be observed. Rouchaud et.al. [2] observed that chlorfenvinphos degradation in soil enriched with organic matter was higher than the

degradation in the control soils. Further a high organic matter content will increase the soil microbial activity and the microbial degradation of the pesticide will be simultaneously increased. Therefore, under field conditions the organic matter content continuously added by the passage of cattle through the dips is an important factor in the pesticide degradation. The radioactivity present in the extract from the styrofoam plugs was very low, so no volatile compounds eseemed to have been formed.

The metabolites identified and quantified in the water extracts using GC/ECD were 2,4-dichloroacetophenone and 2,4-dichlorobenzaldehyde in the following concentrations:

Table I. Cumulative percent of the applied ^{14}C-chlorfenvinphos evolved as $^{14}CO_2$ and trapped in biometer flasks.

Sampling Period (weeks)	Biometer flasks 1 X	Biometer flasks 5X
1	0.1	0.1
2	0.2	0.3
3	0.7	0.9
4	1.2	1.6
5	2.0	2.9
6	2.0	4.1
7	2.1	5.0
8	2.3	5.6
9	2.4	6.1
10	2.4	6.6

Biometer flask	2,4-dichlorobenzaldehyde ng/µl	2,4 dichloroacetophenone ng/µl
1X	0.11	0.26
5X	0.18	0.99

These metabolites were also identified in the extract of the sediment. In addition, diethyl phosphate was identified as a metabolite in the water of biometer flasks 5X by TLC.

3.4. Photoisomerization of chlorfenvinphos

The commercial formulation of Chlorfenvinphos contains a mixture of the Z and E isomers, 90% of Z and 10% of E. The Z isomer is more active than the E, as reported by Beynon et. al. [3]. The GC analyses of the extracts from the model dips showed a change in the isomer proportion with time; for this reason we performed a photoisomerization test with chlorfenvinphos (analytical standard) by exposing it to the sunlight, and analyzed by GC (FPD) the results showed a conversion from the Z to the E isomer (Fig.5). A similar isomerization was found by Beynon and Wright [4] when they applied a formulation of chlorfenvinphos to cabbage leaves.

3.5. Stability of chlofenvinphos

The activity obtained from all the water buffered solutions did not change during the entire sampling time (14 d) at different pHs (range 4-9), the extracts were analyzed by radio TLC and autoradiography, which showed only chlorfenvinphos present at all pHs and times tested. Those results were confirmed by conventional techniques (GC/FPD) and the concentration of chlorfenvinphos determined at the beginning of the experiment was the same determined at the end at all pHs range.

3.6. Leaching of chlorfenvinphos in soil columns

The dips emulsion leachate from the buffer and copper columns were 20.8 and 26.0 % of the initial activity applied to the columns, that showed that a high percentage of the pesticide was retained in the soil and it was eliminated slowly when the simulate rains were applied. After the simulate rains,the pesticide leachate from the buffer and copper columns were 1.8% and 4.3% of the initial applied doses. Therefore, if those values are added to the leachate values from the emulsion dips a total leachate of 22.6% for the buffer columns and 30.3% for the copper columns are produced. These results are present in Figure 6.

The ^{14}C Chlorfenvinphos retained by the soil column was analysed by cutting the column in four sections (Fig. 7). The highest concentration was found in the first section of both type of columns. The high organic matter content present in that section may have influenced the retention of the pesticide. Organic matter in the soil is known to play an important role in the fate of pesticides in soil. The soil in the first section showed more extractable residues (79 %), which can contribute to the dissipation of this pesticide through processes such as volatilization and photodegradation. Inch et al [5] studied the mobility of some organophosphurus insecticides in soil and found that chlorfenvinphos was only slightly mobile. The results of our experiments were consistent with their finding.

The fourth section had a lower quantity of pesticide than the other sections, but the bound residues present were higher. A high quantity of clay was observed in this section and some research performed before in the laboratory showed that vinylphosphates had a high affinity for clays, so this could be the reason why more bound residues were determined.
Total percentages recovery of Cholfenvinphos from the initial activity applied were 69.7% for the buffer column and 62.3% for the copper column, that diference can be explained for the influence of volatilization during the experiment.

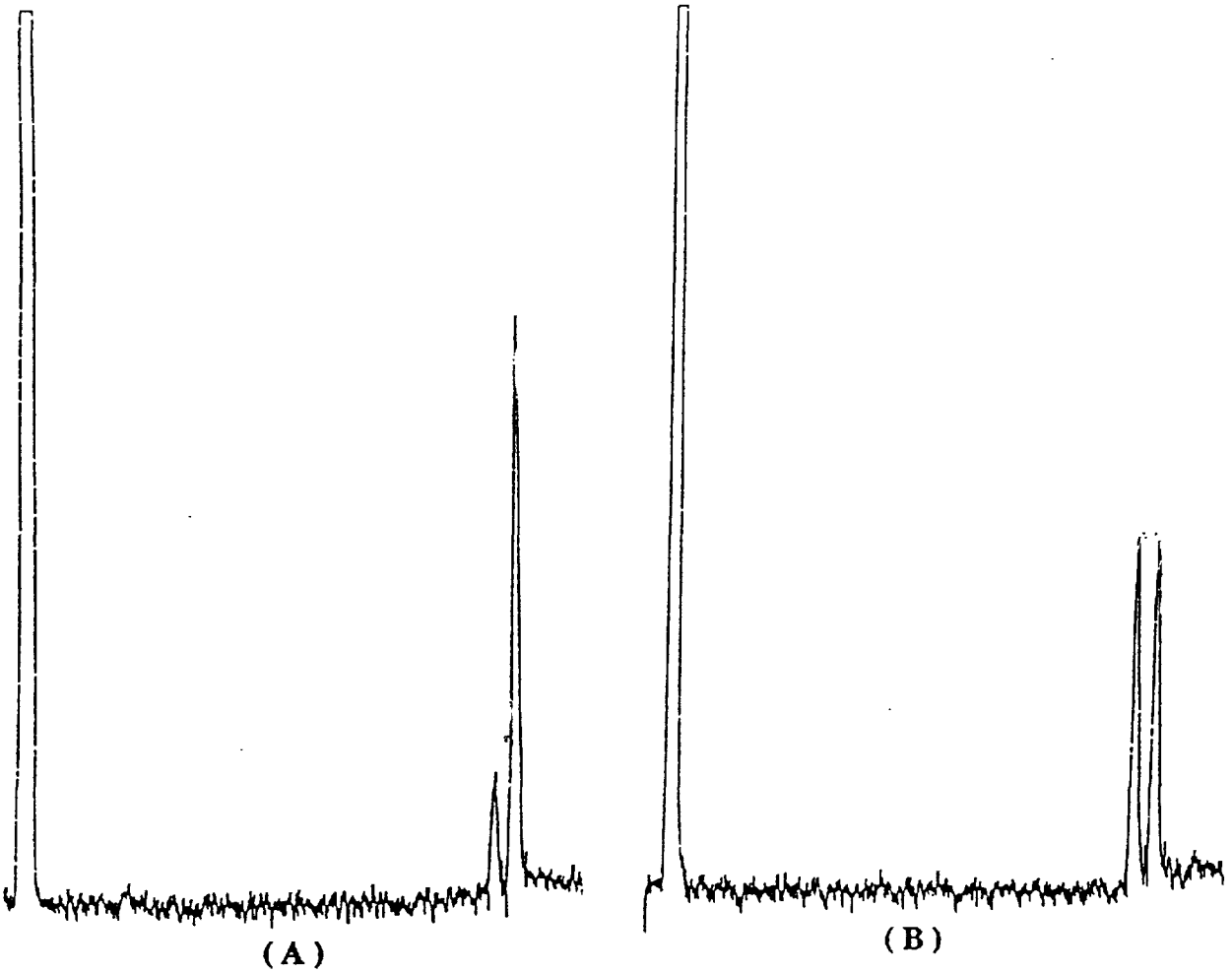
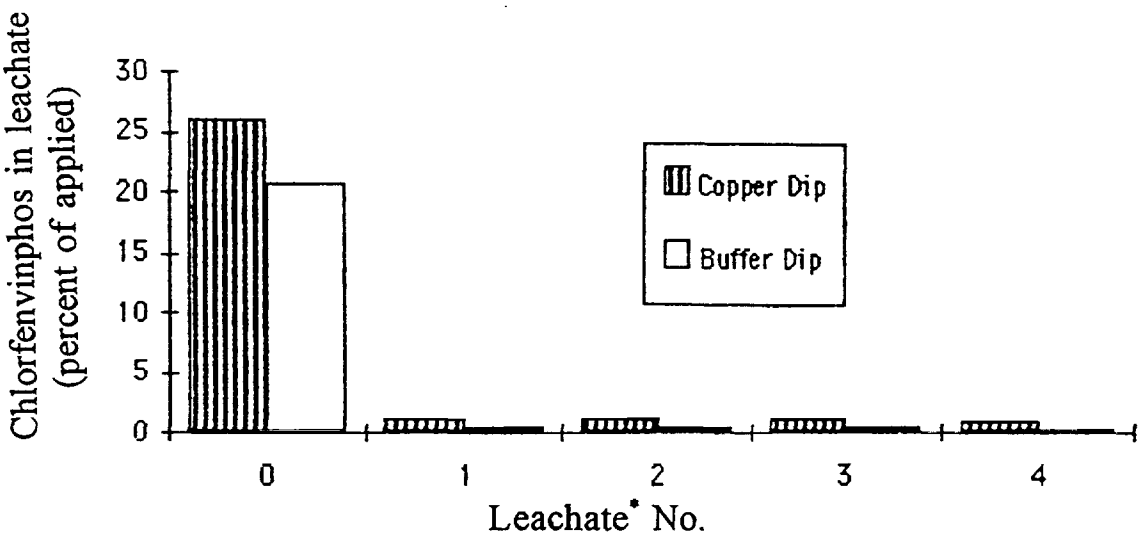


Fig. 5. Gas chromatogram (GC-FPD) from the analysis of chlorfenvinphos without (A) and with (B) exposure to sunlight for 8 hours. (Retention time for E isomer was 9.9 and for Z isomer 10.3 min.).



*0: Leachate from applied suspension
1-4: Leachate from application of simulated rain

Fig. 6. Percent of the applied amount of chlorfenvinphos leached through the soil columns receiveing acaricide suspension amended with superphosphate fertilizer (buffer dip) or copper sulphate (copper dip).

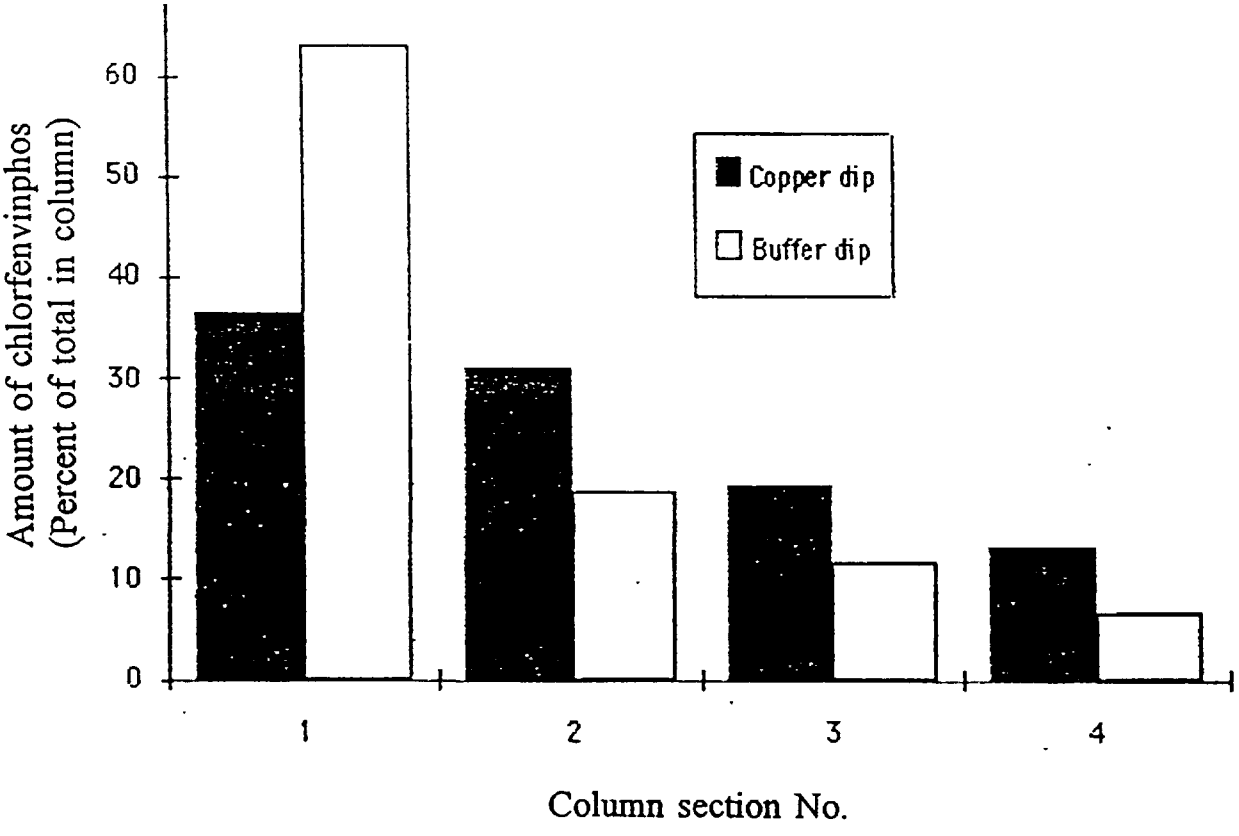


Fig. 7. Concentration of chlorfenvinphos in different 5 cm sections of the soil column

4. CONCLUSIONS AND RECOMENDATIONS

- The principal factor that influence the dissipation of Chlorfenvinphos is volatilization.
- The concentration of the degradation products in the model dips was minimum, and the products identified in the model dips were 2,4 dichloroacetophenone and 2,4 dichlorobenzaldehide at trace levels.
- The monthly recharge at 10% of initial concentration was insufficient to maintain a concentration that would be effective for tick control. An assay using a higher recharge concentration or a shorter recharge frequency should contribute to obtain information to set develop new guidelines for cattle dip usage.
- The addition of superphosphate fertilizer to the dips enhanced the stability of the acaricide, but not enough to maintain the concentration at an effective level. However, the addition of the fertilizer seems to maintain a concentration equivalent to the recharge, an assay using both fertilizer and recharge in the same dip should provide useful information.
- Bound sediment residues increase with time in the dips and in the biometer flasks . However bound residues in biometer flasks are higher, probably by the anaerobic conditions present.
- The micro-organisms from water and sediment influence the mineralization of Chlorfenvinphos. Microbial identification and quantification should be performed in future studies.
- Chlorfenvinphos isomer Z was converted to E by sunlight and it reduces the toxicity of the pesticide, because the Z isomer is less toxic. To increase the useful life of the dip,

exposure to sunlight should be minimized. Photodegradation could be used as a method for the disposal of spent dip waste.

- Under laboratory conditions, chlorfenvinphos is stable for 14 days at pH 4 to 9.
- Chlorfenvinphos showed to be slightly mobile in soil columns, therefore it is not a potential pollutant of the ground water.

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STABILIZATION OF CHLORFENVINPHOS IN MODEL DIPS USING A BUFFER AND A BACTERIOSTAT AGENT

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Abstract

The stability of chlorfenvinphos acaricide was studied in 50 L model dip tanks using a mixture of ^{14}C-labelled and unlabelled chlorfenvinphos with or without recharge. Calcium superphosphate fertilizer was used as buffer and to reduce the pH to below 5, while copper sulfate was used as a bacteriostat. In addition 50 g of sediment from around the dip tank was added to the vats and contents of vats were made up to 50 L using the water available at chalimbana NCSR farm. The buffer, by itself as well as in combination with copper sulfate stabilized chlorfenvinphos in the suspension. Binding with the sediment was a key factor in the reduction of chlorfenvinphos level, which was significantly reduced in all vats that contained sediments. Most of the acaricide bound to the sediment was not extractable. The pH in vats was maintained at 4.8 - 5.0 by the addition of superphosphate fertilizer, which reduced the degradation of chlorfenvinphos. Copper sulfate had no effect on the pH of the suspension and the vats treated with the bacteriostat had the lowest levels of chlorfenvinphos. TLC analysis also showed that acaricide breakdown was greater in non-buffered than in buffered vats. A metabolite, 2,4-dichloroacetophenone, was detected, although at a low level. A study of the leaching potential of chlorfenvinphos in soil columns showed that only 3% of the applied acaricide percolated through the 30 cm length of the column, while most of it remained soil-bound. A colorimetric method for measuring the concentration of chlorfenvinphos in dips was evaluated. The method is based on the reaction of the acaricide with 4-(4-nitrobenzyl) pyridine and subsequent measurement of the intensity of the colour formed with tetraethylene pentamine. The measurement was relatively accurate.

1. INTRODUCTION

Sub-strength concentration of the acaricides in cattle dips has been recognized as one of the factors responsible for development of tick resistance to acaricides. In addition, the presence of sediment and rise in pH are also known as major factors in the loss of the acaricide. The main objectives of the present study were to evaluate the stability of acaricides in livestock dips under current use practices and to develop procedures to improve their stability. Therefore, the affect of a buffering and a bacteriostatic agent and the addition of sediment on the dissipation of chlorfenvinphos was studied. The leaching characteristics of the acaricide in soil were studied in order to evaluate the current dip waste disposal procedures. Another objective was to develop simplified colorimetric methods for rapid measurement of acaricide concentrations in dip vats.

2. MATERIALS

^{14}C-labelled chlorfenvinphos had a specific activity of 1MBq/mg and a benzene solution containing 18.5 MBq in 650 µL was prepared. Other materials used in this work included calcium superphosphate [$Ca_3(H_2PO_4)_2$] fertilizer, containing 46% total phosphate, copper sulfate and Superdip® acaricide (100% chlorfenvinphos). Vats (4 x 50 L) were made of fiber glass. Sediment added to the vats was collected in the proximity of the Chalimbana dip tank. Solvent system used for the development of TLC plates consisted of dichloromethane + acetonitrile + n-hexane (3+3+8, v/v/v).

3. METHODS

^{14}C-Chlorfenvinphos (100 μCi/μl) stock solution was dissolved in 5 - 10 mL dry acetone and mixed with 50 ml formulation containing 25 g non-labelled chlorfenvinphos. The mixture was diluted to 70 ml and stirred for 20 minutes. Cold chlorfenvinphos was added to vats to get a final concentration of 500 mg/L, which was equivalent to the concentration used in the field. The mixture was quantitatively transferred to each 50 L vat and water used for Chalimbana dip tank was used to fill up the vats. The level of the water in the vat was marked.

Copper sulfate was added to vat-1 at the rate of 1 g/L. Super phosphate fertilizer was added to the vat-2 at the rate of 10 g/L. Vat-3 was treated with 500 g of super phosphate fertilizer and 10 g of copper sulfate. Vat-4 was also treated with 500 g of super phosphate fertilizer. Sediment (50 g) was added to vats 1-3 two weeks after the start of the experiment and thereafter fortnightly and monthly in vats with and without recharge, respectively. No sediment was added to vat-4 and it served as the control in both experiments (with or without recharge). The temperature of the vats was recorded by immersing a thermometer at the centre of the vat.

3.1. Sampling:

Before collection of samples, the solutions in the vats were thoroughly mixed and duplicate 25 mL samples were collected from a depth of 20 - 30 cm at the centre of each vat. Samples taken after the initial charge represented the zero time reading (100% activity).Two weeks later, the water level in the vats was refilled to the 50 L mark, after which 50 g of air dried sediment was added, stirred for several minutes and immediately duplicate 25 ml samples were collected. This was repeated every two weeks in case of no recharge or monthly in case of recharged vats until the concentration of the parent acaricide was reduced to 10 - 20% of the initial concentration. The pH of the water before addition of the buffer, bacteriostat and the acaricide was measured.

The second phase of this experiment involved recharging of the vats with the radiolabelled acaricide. This was done by removing 10% of the 50 L suspension from the vat and subsequently replenishing the vat by adding 10% of the initial concentration of the acaricide. Five litre samples collected from the respective vats were filtered and the sediment was dried at 50°C for three days to a constant weight. The sediment was weighed and extracted with methanol using a soxhlet extraction system. The extract was concentrated. Scintillation fluid was added and the radioactivity determined.

3.2. Leaching test

Core soil samples were collected up to a depth of 30 cm. The soil was air dried, homogenized and sieved through a 2 mm mesh sieve. Three glass columns (25 x 120 mm) were packed, each first with 0.5 g macerated filter paper and then with 30 g dried soil. The columns were closed with a rubber stopper through which a 50 mm long glass tube was inserted. The soil was conditioned by passing 100 mL portions of distilled water through it and then allowed to drain. They were kept for two days in the laboratory at ambient temperatures and then 4 mL suspension of ^{14}C-chlorfenvinphos was added to the top of each column. Distilled water (2.5 L) was added to the top of each column to simulate 2 mm of rainfall. The radioactivity in the eluting water was determined by LSC according to the method of Gonzalez et al [1].

3.3. Analysis of chlorfenvinphos in the vats

Total chlorfenvinphos concentration in the vats was estimated by taking triplicate samples from well mixed suspension in the vats and measuring radioactivity in a Packard 1000 liquid scintillation counter. The suspension samples (1 mL) were also centrifuged at 3000 rpm for five minutes and the radioactivity in the supernatent measured. Radioactivity in sediment was calculated by substracting the radioactivity found in the supermatent from the total radioactivity added to the vat. A sample of the suspension was extracted with acetonitrile, and the organic layer concentrated and used for identification of chlorfenvinphos and its metabolites by TLC. Bound residues were determined by filtering 5 L vat samples collected monthly and the sediment obtained, dried and soxhlet extracted with methanol for four hours. The methanol extract was used for the estimation of the total extractable ^{14}C and for analysis by TLC. Residues remaining in the sediment after Soxhlet extraction were not estimated.

3.4. Evaluation of a colorimetric method of analysis

A colorimetric method used by Noble et al [2] for the estimation of dimethoate residues in fruits and vegetables was adopted for use in the estimation of chlorfenvinphos concentration in the dips. The method is based on the alkylating properties of organophosphate insecticides by their reaction with 4-(4-nitrobenzyl) pyridine at high temperatures to form the dye precursor. Colour develops on the addition of tetraethylenepentamine. A 100 µL aliquot of a five percent solution of 4-(4-nitrobenzyl) pyridine in acetone and 100 µL of 0.4% oxalic acid were pipetted into a 20 ml test tube. One ml chlorfenvinphos standard (853 mg/L) was then added to the tube and the contents heated at 100°C for one hour in a waterbath. To the cooled test tube 5 mL of 10% (v/v) tetraethylenepentamine in acetone was added. The absorbance was measured at wavelengths ranging from 430 nm to 710 nm in a Corning colorimeter .

4. RESULTS AND DISCUSSION

4.1. Vats without recharge

The initial pH of the suspension was 7.2 and was lowered to 5.5 with the addition of copper sulfate, to 5.2 with the addition of superphosphate and to 4.6 when both were added. However, the pH of the suspension in the vat treated with only copper sulfate steadily increased to 8.0 with time; whereas, the pH in vats which were treated with only superphosphate changed slightly to 4.6 as shown in Fig. 1a. Similarly, in vats that were treated with copper sulfate and superphosphate the pH changed slightly to 5.7.

As shown in Figs. 2 a & b the radioactivity in the control vat (vat-4) dropped to 73.2 % of the initial concentration after 404 d. However, the radioactivity in the vats treated with superphosphate, copper sulfate and both decreased to 29.1, 15.3 and 31.7 %, respectively. These data indicate that the smallest loss of radioactivity was in vat-4 which did not have any added sediment. Also, vat-2, which had added copper sulfate but no superphosphate, had a greater loss of radioactivity than the other vats all of which had added superphosphate, indicating that the addition of superphosphate fertilizer can stabilize chlorfenvinphos in animal dips.

The half life of ^{14}C-chlorfenvinphos in the suspension in the vats was calculated to be 110 d in vats treated with copper sulfate, 193 d in vats treated with superphosphate and 313

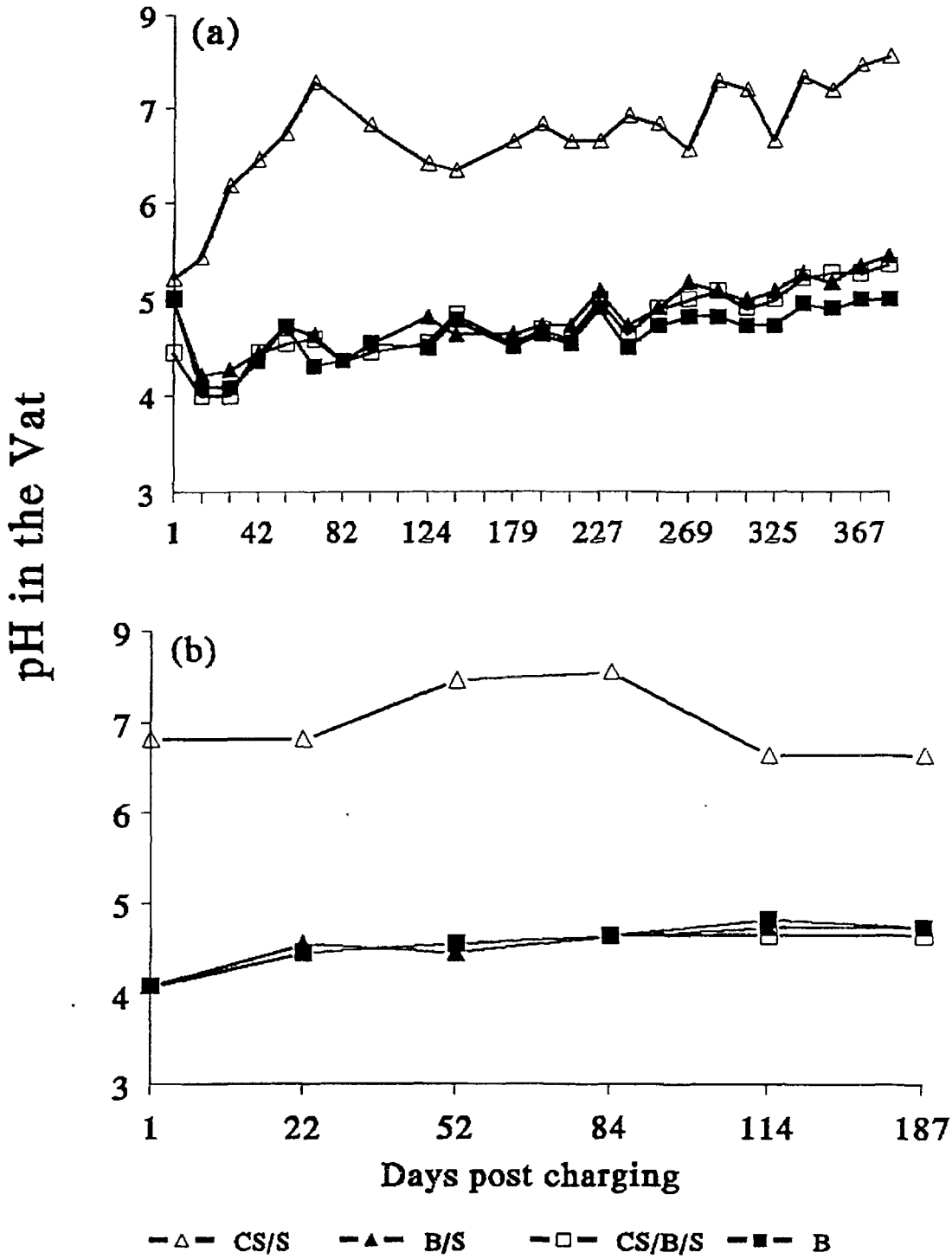

FIGURE 1: Changes in pH with time in model dip vats with no recharge (a) and with recharge (b).

CS = Copper Sulphate B = Buffer

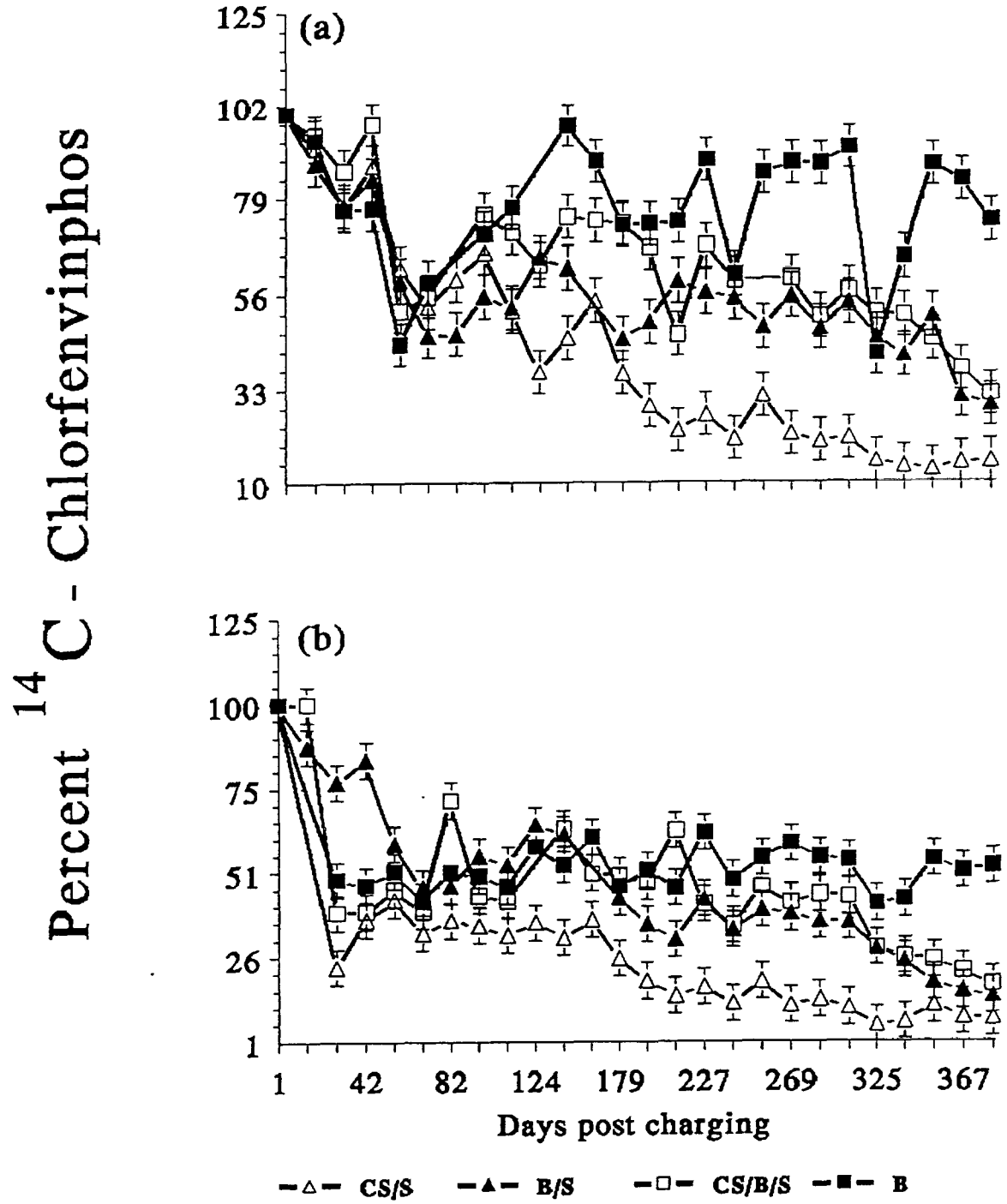

FIGURE 2: Levels of ^{14}C-Chlorfenvinphos in model dip vats with time under the no recharge system in whole vat (a) and Supernatant samples (b).

CS = Copper Sulphate B = Buffer

d in vats treated with both additives. It is evident that in the absence of recharge the use of calcium superphosphate reduced chlorfenvinphos degradation.

4.2. Vats with monthly recharge

When the superphosphate treated vats were recharged on a monthly basis the pH remained below 5 during the 210 d period of the experiment (Fig. 1b). However, in the case of the vat receiving only copper sulphate the pH fluctuated around 7 during the same period.

Figs. 3a & b show that the radioactivity in the control vat dropped to 91.8 % in 187 days. During the same perriod radioactivity in the vats receiving superphosphate, copper sulfate and the mixture of the two dropped to 78.8, 52.8 and 65.8 %, respectively. The half life of ^{14}C- chlorfenvinphos was extended to more than 210 d in all cases except in the vat treated with copper sulfate and sediment only, in which case the the half life was 187 days. Thefore, the half life period of chlorfenvinphos in the vats receiving recharge was longer than in the vats not receiving recharge.

The pH of the suspension appeared to be a key factor affecting the stability of chlorfenvinphos because degradation was enhanced in the vats where the buffer was not added and the pH remained at 7 or above. In vats where the buffer was added the pH remained around 5 and the stability of ^{14}C- chlorfenvinphos was much improved.

The temperature of the vats is shown in Figs. 4 a & b. It ranged from 13-18 °C during the cold season (May - August), 21-22 °C during the hot season (September - December) and 19-21 °C during the rainy season (January - April). The temperature of the dip suspension did not seem to affect the stability of ^{14}C-chlorfenvinphos.

4.3. TLC analysis

The degradation of the acaricide detectable first by TLC was observered on samples taken 138 days after the treatment. This occurred in the vat which had received only copper sulfate and no superphosphate. A total of 77.4 % of the total spotted activity was recovered from the spot with an Rf value of 0.29, corresponding to that of chlorfenvinphos standard. Unidentified breakdown products with Rf values of 0.05, 0.19 and 0.49 accounted for 5.8, 10.7 and 4.1 % of the activity, respectively. The remaining 1.9 % of the activity corresponded with the standard for 2,4-dichloroacetophenone, a chlorfenvinphos metabolite with Rf of 0.58.

In vat-2 which received superphosphate and sediment, TLC analysisof the suspension extract showed that most of the radioactivity corresponded with the spot for chlorfenvinphos and very little breakdown had occurred. The spot for chlorfenvinphos at Rf 0.29 accounted for 97.5 % of the radioactivity applied to the plate. Only one other small radioactive spot, possibly for an unidentified metabolite, was present at Rf 0.50 and accounted for the remaining 2.5 % of the radioactivity.

In vat-3, which received superphosphate, copper sulfate and sediment, 89.6 % of the recovered activity corresponded with the spot for chlorfenvinphos. Two other spots for unidentified metabloites at Rf 0.15 and 0.13 accounted for 5.1 and 5.0 % of the radioactivty, respectively. A fourth spot with 0.19 % of the radioactivity was present at Rf 0.56 and it cochromatographed with the spot for 2,4-dichlorobenzophenone.

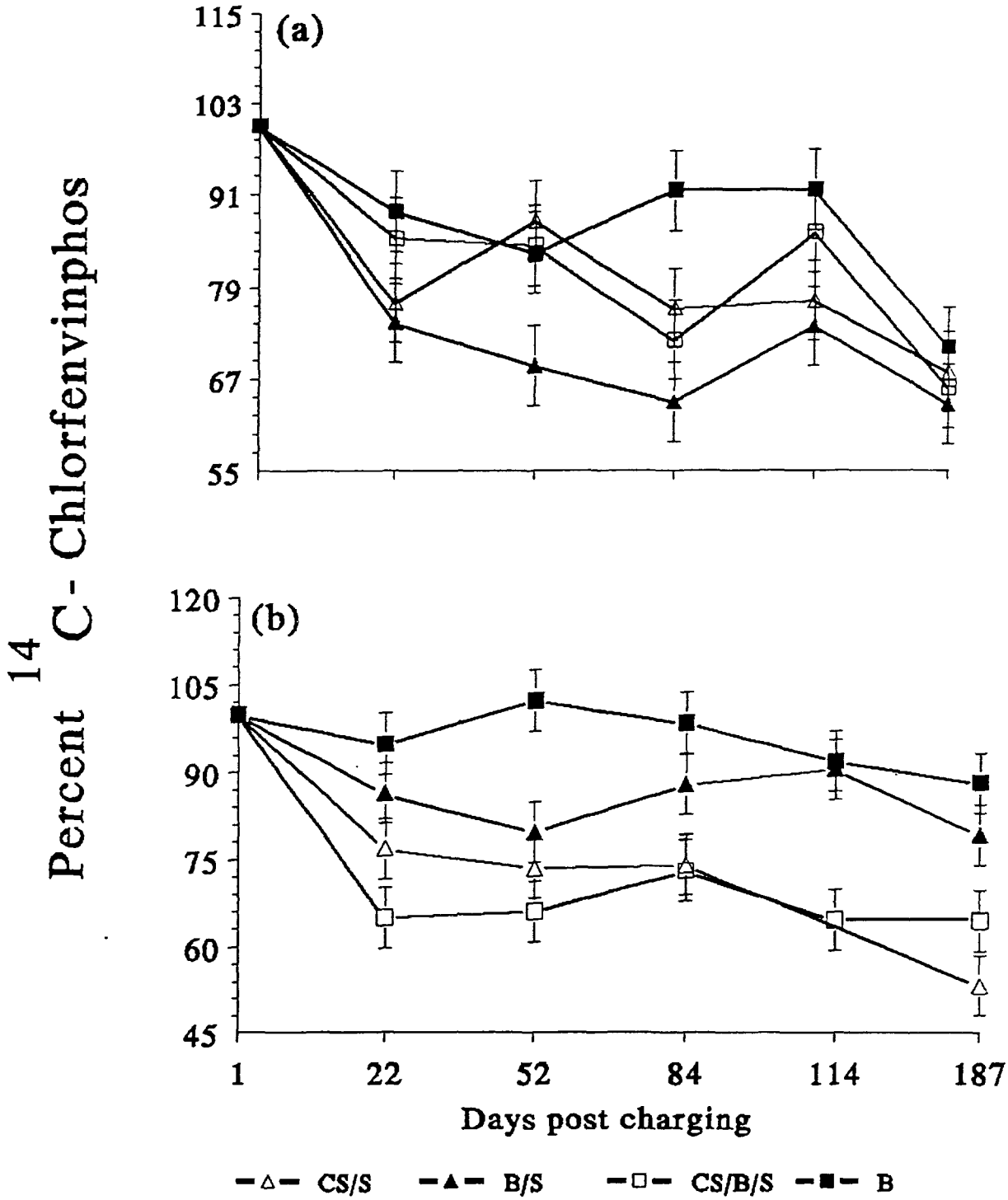

FIGURE 3: Levels of ^{14}C-Chlorfenvinphos in model dip vats with time under recharge in whole vat (a) and supernatant samples (b).

CS = Copper Sulphate B = Buffer

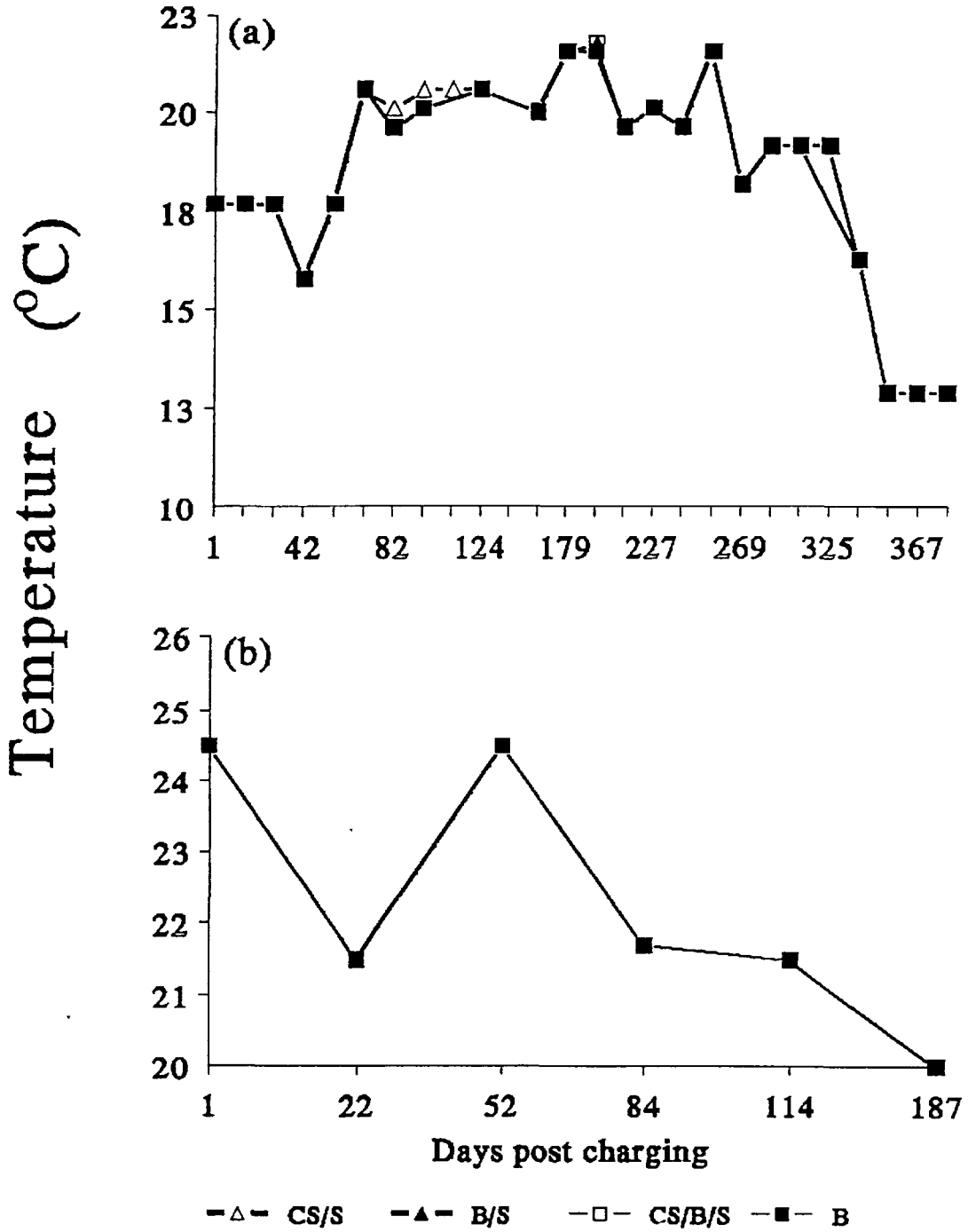

FIGURE 4: Changes in temperature in model dip vats under no recharge (a) and recharge (b).

CS = Copper Sulphate B = Buffer

For vat-4, which received only superphosphate, very little degradation was observed and 93.3 % of the recovered activity was associated with chlorfenvinphos. Two other spots for unidentified metabolites accounted for 5.1 and 1.6 % of the radioactivity. No radioactive spot corresponding with 2,4-dichlorobenzophenone was detected. The results also indicate that the addition of superphosphate to dips containing chlorfenvinphos will improve the stability of the

acaricide. McDougall and Machin [3] also reported that the carbamate acaricide promacyl was stabilized in cattle dips by the addition of copper sulfate and magnesium chloride and that its breakdown was reduced by lowering the pH to 5.0.

4.4. Leaching of chlorfenvinphos in soil

The characteristics of the soil used are shown in Table I. The soil had high calcium, magnesium and carbon content. These elements indicate that the soil has a high clay content and, therefore, expected to have high adsorptivity characteristics. It was, therefore, expected that practically all ^{14}C-chlorfenvinphos would bind with the soil and would not leach through.

Table I: Physical-chemical characteristics of the soil from Mt. Makulu Research Centre.

Elements	Content (%)
Calcium	10.3
Magnesium	1.0
Potassium	0.8
Sodium	0.1
Carbon	1.7
pH	7.8
^{14}C-Chlorfenvinphos (%) leached (mean of three replicates)	3.1

As expected, the results obtained from leaching experiment showed that very little radioactivity (3.1% of the applied) percolated through the soil column.

4.5. Test of colorimetric method

The absorbance values observed for the various wavelengths are shown in the Table II. The absorbance maximum for chlorfenvinphos was 540 nm.

Table II. Absorbance of chlorfenvinphos at different wavelengths:

Wavelength (nm)	430	470	490	520	540	580	710
Absorbance (%)	0	0	0	0	0.07	0.02	0

Chlorfenvinphos samples were prepared as described above and heated at 100°C in a waterbath for periods ranging from 10-120 minutes. Colour was then developed with tetraethylene pentamine and absorbance for each sample determined at 540 nm. The results on the optimum reaction time and absorbances are shown in Table III.

Table III: The relationship between the reaction time and absorbance at 540 nm.

Reaction Time (min)	A_{540} {% Toluene)	A_{540} (% Acetone)
10	0.01	0.06
20	0.05	0.09
30	0.06	0.12
40	0.01	0.10
50	0.03	0.15
60	0.12	0.17
90	0.07	0.10
120	0.11	0.13

Although reaction for 60 minutes gave the greatest absorbance, 30 minute also gave adequate absorbance. Therefore, in order to save time and make the method rapid, reaction was carried out for 30 minutes. Reaction in acetone and toluene was compared. Reaction in toluene, despite lower absorbance values, gave more reproducible results. Samples of chlorfenvinphos at known concentrations were tested and gave relatively accurate readings as shown in Table IV. Therefore, this method can be used in monitoring of chlorfenvinphos concentration in cattle dips in the field.

Table IV. Observed concentrations of test samples

Samples	Actual Concentration	A_{540}	Observed Concentration
A	200 mg/L	0.161	198 mg/L
B	300 mg/L	0.181	312 mg/L
C	500 mg/L	0.223	416 mg/L

5. CONCLUSION AND RECOMMENDATIONS

This study on procedures to stabilize ^{14}C-Chlorfenvinphos in cattle dips using nuclear techniques has shown that, in the absence of recharge, the breakdown of the acaricide slowed down when superphosphate fertilizer was added to the suspension in the dip. Sedimentation played a major role in binding and removing some of chlorfenvinphos from the solution. Recharge of the vats with the acaricide at the rate of 10 % of the original concentration on a fortnightly or monthly basis, failed to maintain the acaricide at 100% levels. This may be attributed to the role of sediment in binding the acaricide and removing it from solution. Soil binding, on the hand, reduced the mobility of the acaricide through the soil and hazard of contamination of the ground water from the disposal of acaricide dip waste.

The following recommendations are made:

i. Calcium superphosphate fertilizer should be used, alone or in combination with a bacteriostat, to stabilize chlorfenvinphos in animal dips.
ii. The presence of sediment in the vats should be reduced to improve the amount of acaricide available in solution
iii. These results should be validated in actual field dips and the cost vs benefit ratio for the farmer/rancher determined.

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PROTOCOL FOR THE MICROBIAL DEGRADATION OF COUMAPHOS FROM CATTLE DIP

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Abstract

Insecticide wastes generated from livestock dipping operations are well suited for biodegradation processes since these wastes are concentrated, contained, and have no other significant toxic components. About 400,000 L of cattle dip wastes containing approximately 1500 mg/L of the organophosphate coumaphos are generated yearly along the Mexican border from a USDA program designed to control disease carrying cattle ticks. Use of unlined evaporation pits for the disposal of these wastes has resulted in highly contaminated soils underlying these sites. Previous work has shown that microbial consortia present in selected dip wastes can be induced to mineralize coumaphos. Our laboratory results show that these consortia are able to colonize plastic fibers in trickling biofilters and can be used in these filters to quickly metabolize coumaphos from dip wastes. A field scale biofilter capable of treating 15,000 litre batches of dip waste was used to reduce the coumaphos concentration in two successive 11,000 litre batch trials from 2000 mg/L to 10 mg/L in approximately 14 d.

1. INTRODUCTION

Research at the USDA's Agricultural Research Service (ARS) has shown that cattle dips from a variety of locations contain bacteria that, given the appropriate conditions, are able to metabolize coumaphos in the used dips [1,2,3,4]. The complete degradation of the coumaphos (to carbon dioxide, chloride, bacterial biomass, and water) is a multistep process and is carried out by consortia of bacteria rather than by a single type of bacteria. Although the origins of these bacteria are unknown, they are likely to be natural soil bacteria that are carried in by the animals being dipped.

The conditions necessary for the coumaphos degrading bacteria for growth include adequate concentrations of nutrients and oxygen, adequate temperature, control of the pH of the solution, and mixing of the solution to keep the coumaphos suspended and available for the organisms to degrade it. Experiments at the USDA show that most used dips have adequate nutrient concentrations (from the cow manure) for bacterial growth. In addition, many dips have been amended with triple super-phosphate fertilizer to limit potasan formation during their normal use [5]. During the coumaphos degradation process, this triple super-phosphate serves as very good pH buffer as well as a potential phosphate source for the bacteria. Thus, if the dip already contains the necessary bacterial consortia for coumaphos metabolism and the temperature is within the range needed for bacterial growth (15°- 35° C), all that is normally needed during the biodegradation process is to: 1) adjust and maintain the pH of the dip to a biological range (pH 7-8.5), 2) provide oxygen to the bacteria, and 3) make the coumaphos particles available to the bacteria.

2. SETUP AND OPERATION OF THE BIOFILTER UNIT

Recent USDA research on developing simple systems for coumaphos biodegradation has focused on the use of trickling biofilters similar to those used in many municipal wastewater treatment systems [6]. In this system the dip to be treated is pumped into a fiberglass storage tank that has been modified for this use. If the dip has already been amended with super triple phosphate, then the pH of the dip is adjusted and maintained at pH 7.5-8.0 by the addition of sodium hydroxide pellets. If the dip has not already been amended with super triple phosphate, then either super triple phosphate (11 kg/ 3700 L) or sodium bicarbonate must be added to provide a pH buffer, and the pH of the dip is adjusted and maintained at pH 7.5-8.0 by the addition of sodium hydroxide pellets. In USDA field trials, approximately 13 kg of sodium hydroxide was needed to initially adjust the pH of each 15,000 L batch of dip that had been previously amended with super triple phosphate. The dip is then continuously pumped from the bottom of the tank (at a flow rate of about 370 L/min) up to the top of the tank and over a material (the biofilter support) to which the coumaphos degrading bacteria can attach and grow. Multiple layers of a lightweight plastic (polyethylene) mesh pad work well as the biofilter support. After passing through the mesh pad, the dip then falls through a grating back to the reservoir below (and is oxygenated during the process). Bacteria in the dip attach to the pad, capture and degrade the coumaphos (and other organic compounds) from the dip solution passing by. The passing solution also supplies oxygen to the coumaphos degrading bacteria.

For the first use of a new biofilter unit it is important that the dip being treated contain the necessary bacterial consortia for complete coumaphos degradation. Alternately, the dip can be inoculated with another dip that does contain the proper consortia or the biofilter pad can be inoculated using a used biofilter pad from another location. Once the consortia has attached to the biofilter pad, no further inoculation is necessary. In addition, it is not necessary for subsequent dips to contain the necessary consortia.

As the coumaphos and other organic compounds in the dip are metabolized by biofilter bacteria, chloride and small organic acids are generated. This leads to a decrease in the pH of the dip solution. To maintain proper conditions for the biofilter sodium hydroxide pellets are added every 48-72 h as needed to the dip solution to maintain a pH of 7.5-8.0. Automatic pH control equipment using concentrated solutions of sodium hydroxide could be used in lieu of manually adding the solid material.

After 2-3 batches of waste dip (15,000 L each) the biofilter support will foul with excessive bacterial growth and the silt present in the spent dip. When this happens, the mesh pads can be washed out and reused. When the biofilter becomes clogged, coumaphos will become trapped in the biofilter without being degraded. Thus, the effluent resulting from washing the pads will contain undegraded coumaphos and should be directed back into the tank reservoir. It is possible to simply climb into the top of the tank (wearing appropriate protective clothing) and wash out the pads *in situ* by lifting them up while spraying them with the waste dip solution (or clean water). The pads can then be rearranged back on top of the grating and the system restarted to degrade the residual coumaphos that was released by the biofilter washing. After coumaphos levels in the dip have reached the target treatment levels, the biofilter pads may be washed again and the tank emptied before a new batch of dip is loaded.

3. ANALYSIS OF BIOFILTER OPERATION

In USDA field trials, 100 mL samples of treated dips were sent by overnight mail to a laboratory for coumaphos analysis by high performance liquid chromatography (HPLC) [1]. Before sampling the biofilter reservoir, the reservoir was vigorously aerated to resuspend coumaphos particles that had collected on the bottom and sides of the tank. Prior to HPLC analysis in the laboratory, aliquots from these samples were either diluted five-fold with methanol (for samples containing levels of coumaphos above 30 mg/L) or concentrated after extraction with equal volumes of ethyl acetate (for samples containing levels of coumaphos below 30 mg/L).

In the field, the progress of the biofilter toward degrading coumaphos in the dip can be assessed by noting the amounts of sodium hydroxide necessary to maintain a pH range of 7.5-8.0 in the treated dip. In USDA field trials, approximately 12 kg of sodium hydroxide pellets were added to each batch of biofilter treated dip during the treatment period. After the bulk of the coumaphos in the dip is degraded, the pH of the dip no longer decreases. Conditions which slow or stop coumaphos degradation by biofilter bacteria (such as temperatures outside of the working range or clogging of the biofilter) also result in a decrease in sodium hydroxide consumption by the biofilter.

4. BIOFILTER PERFORMANCE DATA

Field trials using a USDA biofilter unit were conducted from September to December, 1995. Three batches of waste dip containing coumaphos were treated. The temperatures of the dips ranged between 25-29° C during the first two trials. Figure 1 shows the results of these trials. The first two trials used batches of waste dip containing flowable liquid CoRal^{R} and the last batch used dip containing a wettable powder formulation of CoRal^{R}. In the first two trials, coumaphos levels in the dip decreased two hundred-fold (2000 mg/L to 10 mg/L) within fifteen days. No further significant decrease in coumaphos was observed after longer treatment. During the third trial, the biofilter became visibly clogged with excess bacterial growth and silt. Although coumaphos levels in the dip solution decreased significantly (from 2000 mg/L to about 200 mg/L in 15 d), rinsing the biofilter pads to unclog them released a large amount of undegraded coumaphos that had been trapped on the clogged biofilter. After the system was restarted with the rinsed pads, coumaphos levels in the dip decreased from 1400 mg/L to 900 mg/L in 7 d. An abrupt change in temperature (dropping from 22.5° C to 10° C) significantly slowed further degradation. (not shown). Chloride is released from coumaphos as it is biodegraded and is an excellent soluble indicator of the biodegradation process. Figure 2 shows the stoichiometric increase in chloride concentrations and decrease in coumaphos concentrations during the first two field trials. Figure 3 shows the chloride release results from the third trial. These results suggest that approximately half of the coumaphos was biodegraded before the biofilter fouled, even though the coumaphos concentration in the effluent decreased by more than 95 %.

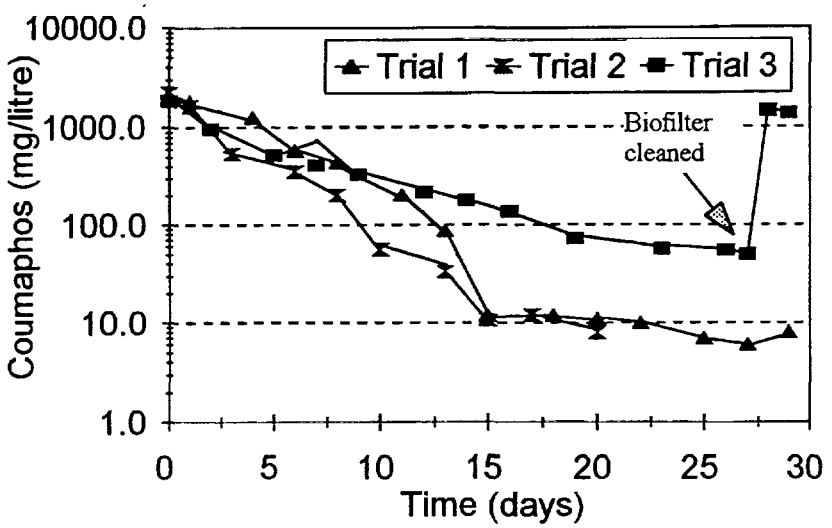

FIG. 1. Removal of coumaphos from three 11,000 litre batches of biofilter treated dip. Dip samples were analyzed by HPLC for coumaphos and its metabolites. In the first two trials, coumaphos concentrations decreased from 2000 mg/litre to 10 mg/litre within 15 days at 30 °C. Additional treatment time did not result in significant decreases in the concentration of coumaphos below 10 mg/litre. No significant levels of aromatic metabolites were detected during the trials. The biofilter pad fouled during the third batch and was cleaned at the time indicated to release bound undegraded coumaphos.

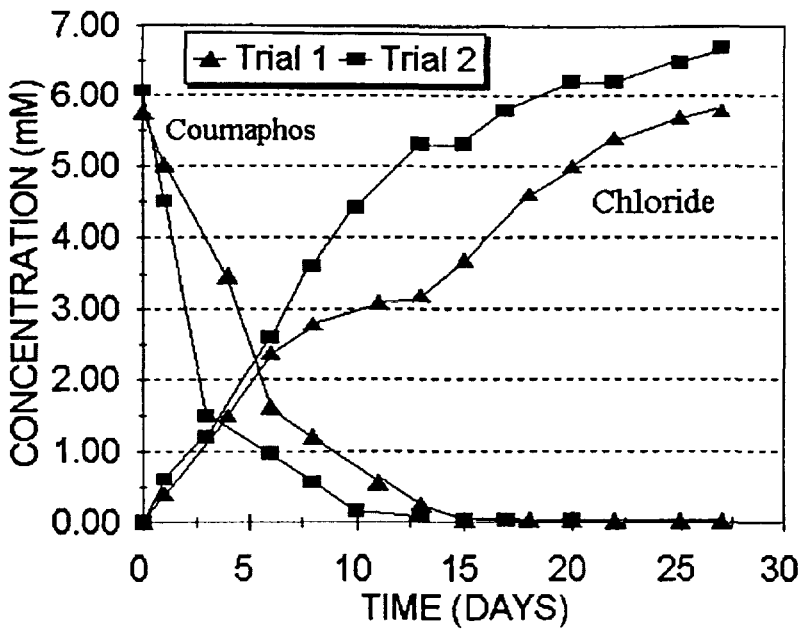

FIG. 2. Release of chloride from coumaphos during biofilter treatment trials 1 &2. During the first two trials, chloride concentrations increased stoichiometrically as the coumaphos concentrations decreased. Increases in chloride levels in the biofilter effluent beyond the expected stoichiometric amounts were due to evaporative water loss during biofilter operation.

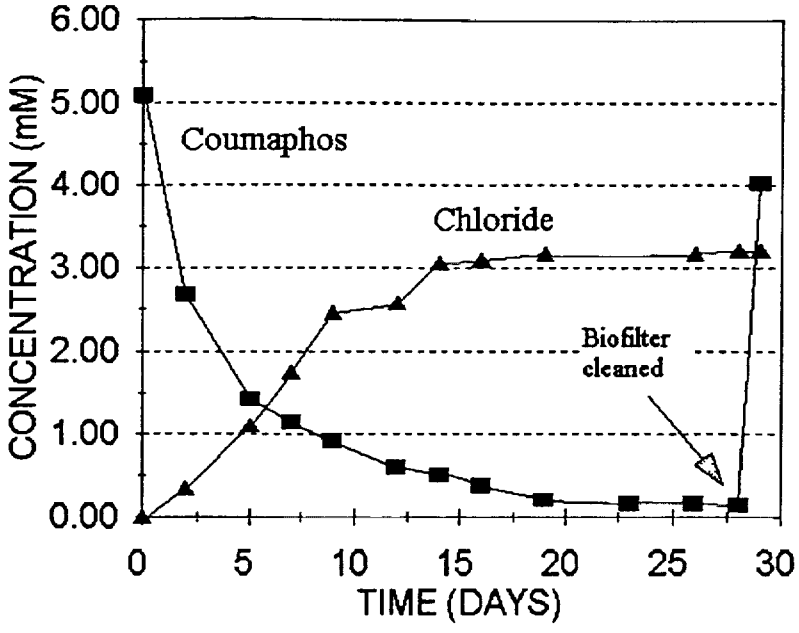

FIG. 3. Release of chloride from coumaphos during biofilter treatment. During the third trial, chloride release was less than stoichiometric because of biofilter fouling. Biofilter cleaning released approximately 1.5 - 2 mM more coumaphos than was expected from the chloride release results. Although evaporative water loss probably accounted for some of the discrepancy between chloride and coumaphos levels, these results suggest that some of the coumaphos from the previous two trials was trapped on the pads without being degraded.

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STUDIES ON THE ENVIRONMENTAL CHEMISTRY AND DISSIPATION OF ^{14}C-LABELLED DELTAMETHRIN AND AMITRAZ IN MODEL DIPS

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Abstract

The degradation of ^{14}C-labelled deltamethrin under variable conditions of pH and temperature, leaching of ^{14}C-labelled amitraz in soil columns and dissipation of these insecticides in model dipping vat was studied. Deltamethrin was stable for 28 d in buffer solutions at pH 4,7 and 8 and at temperature of 14, 28 and 35°C, but degraded in a solution at pH 9. Metabolites identified by TLC corresponded to 3-phenoxybenzoic acid and 3-phenoxybenzyl alcohol. After 75 days of standing in a model dip, about 60% of the total radioactivity was found in the sediment and corresponded mainly to deltamethrin When ^{14}C-Amitraz was applied to soil columns, placed under outdoor conditions, 4 months later all of the radiocarbon was found in the upper 10 cm section and about 90% of the recovered radioactivity corresponded with amitraz A study of the dissipation of amitraz in a field vat showed that after 20 d of use the concentration of amitraz in a field dip decreased from 210 µg/mL to 155 µg/mL.

1. INTRODUCTION

Deltamethrin and amitraz insecticides are used for the control of ectoparasites on livestock and poultry [1,2,3]. In Brazil they have been in use as acaricides for more than 10 years as pour on or in vat solution for the control of the cattle tick, *Boophilus sp.* Amitraz is used on a large scale, and its discharge in the soil is the usual way for disposal of the used dips. The addition of lime to the vats, to increase the pH to about 10 or higher, is a usual procedure recommended by the manufactures to reduce the degradation of amitraz. Information on the chemical behaviour, fate and stability of amitraz and deltamethrin in the dips and underlying soil as well as their sorption to the dip sediment is not available. In this paper we report the results of studies on deltamethrin and amitraz to evaluate their stability in a field vat (amitraz) and in a model vat (deltamethrin) and their behaviour in soil.

2. METHODS AND MATERIALS

^{14}C-deltamethrin [(s)-α- cyano - 3 - phenoxybenzyl (1R, 3R) - cis -3 - (2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate, (7-^{14}C-benzyl labelled)], with specific activity of 1.29 MBq/mg (651 MBq/mmol) and radiochemical purity of 98+% was purchased from International Isotope Munich, Germany. Commercial deltamethrin (Butox) was obtained from the local market in 10 ml ampoules. Analytical deltamethrin (99% purity) was received from the United States Environmental Protection Agency. Standards of metabolites used as reference in chromatography were 3-phenoxybenzoic acid and 3-phenoxybenzyl alcohol.^{14}C-Amitraz [N-methylbis(2,4-xylyliminomethyl)amine] with specific activity of 1.51 MBq/mg was obtained from Institute of Isotopes, Budapest, Hungary. It was purified on polyamide TLC plates before use.

2.1. Study of the effect of pH and temperature on the stability of deltamethrin.

^{14}C-Deltamethrin (50 µL of 111 kBq/mL) and 50 µL Butox (50 g/L E.C. formulation) were added to 5 mL 0.2M phosphate buffer solutions of pH 4, 7, 8 and 9. Solutions were incubated in the dark at 15, 25, 35 and 45°C. Two replicates samples were taken and analysed

at each sampling time. Sample were taken at 0, 3, 4, 7, 14, 28 and 48 days after the treatment and extracted with 3x5 mL of a mixture of hexane+ethyl acetate (1+1, v/v). The organic fractions were combined, filtrated over sodium sulfate, concentrated in a rotoevaporator (Büchi) at 40°C to dryness and redissolved in 5 mL of the solvent mixture. Aliquots (100µl) were radioassayed in a Packard Model 1600TR liquid scintillation counter (LSC) and 200µl applied to a silica-gel thin layer plates (Merck 60 F254). The plates were developed in the solvent mixtures which constituted hexane+ethyl acetate (1+1, v/v) or toluene+hexane+acetic acid (15+3+20, v/v/v). Deltamethrin recovery was 89.5%. Air dried plates were scanned in a Berthold LB 2723 TLC scanner and autoradiographed. Radioactive zones were scraped in to liquid scintillation vials and radioassayed by LSC. The organic solution was also analyzed by high performance liquid chromatograpy (HPLC). For this purpose the organic solution was concentrated under a stream of nitrogen. A Shimadzu Model LC-10 HPLC equipped with a Phenomenex Zorbax silica column (5 µm, 250 mm length x 4.6 mm ID), a Packard radiometric detector and a U.V. detector was used for the analysis. The wavelength of the U.V. detector was fixed at 220 nm. The eluting solvent mixture was comprised of hexane+dioxane (1+1, v/v) and the flow rate was 1 mL/min.

2.2. Degradation of deltamethrin in water+sediment in a biometric flask

A 300 µL solution of ^{14}C-deltamethrin in hexane (1.11 MBq/mL) was evaporated and redissolved in 300µL acetone. To this 500µL Butox was added to obtain a ^{14}C-Butox preparation. A 50µL aliquot of this preparation was added to each of the biometer flasks containing (a) 50 mL pond water, (b) 50 mL pond water plus 80 mg sediment, (c) 50 mL pond water plus 80 mg sediment autoclaved at 120°C for 1 hour for 2 consecutive days and (d) 50 mL pond water plus 800 mg sediment. The evolved $^{14}C0_2$ was estimated on a weekly basis. At the end of 56 d the sediment was separated from the water and extracted 3 times with a hexane+ethyl acetate (1+1, v/v) mixture.

2.3. Dissipation of deltamethrin in a model dip vat under field conditions.

A model dipping vat was prepared by placing a glass tank (50 x 22 x 22 cm) in a wooden box lined with soil between the tank and the box. The vat was placed outdoors and a roof was constructed over it to protect it from the rain and direct sunlight. The vat was filled with 20 L of pond water. A mixture of 10 mL Butox and 4 mL ^{14}C-deltamethrin solution (1.11 MBq/mL) was prepared and added to the water in the vat. Finally, 20 g sediment was added to the vat. Duplicate samples of 10 mL of the water sediment mixture were collected at time zero and every 2 weeks for analysis. Aliquots of 1 mL of the sediment+water mixture were analysed for total ^{14}C by LSC. The rest of the sample was centrifuged and the activity of the supernatant measured.

To estimate deltametrhin residues, the sample was extracted with 3x10 mL hexane+ethyl acetate mixture. The organic phase was combined, concentrated to 5 mL and analysed for deltamethrin and metabolites by TLC. Analytical standard of deltamethrin was used for confirmation of the spot for deltamethrin.

The sediment in the centrifuged samples was washed 3 times with 5 mL of hexane:ethyl acetate (1:1) mixture, the extracts combined, concentrated to 1 mL and analysed by TLC.

2.4. Leaching of amitraz in soil columns

^{14}C-Amitraz solution and 20 mL Traitox (a commercial formulation of amitraz) were added to 10 L pond water in a glass tank and 7 g $Ca(OH)_2$ added to simulate field vat conditions. After 3 months, 3 litre of distilled water was added to the tank to bring the total to the initial volume and the vat contents were used for leaching studies.

In order to prepare soil columns polyethylene cylinders (5 cm dia x 50 cm long) were driven into the soil, which contained 61% clay, 12% silt, 27% sand and 2.9% organic matter and had a pH of 6. The intact soil columns were removed from the field and placed in a plastic box with sand and vermiculite to fill the spaces between the columns and to allow collection of water from each column. A 1 mL aliquot from the vat suspension containing 92.5 kBq ^{14}C-amitraz was applied to the top of each column. Water was added to the columns and the leachate collected at 0, 30, 60 and 180 d after treatment and replicate samples analysed. At the end of 180 d the soil columns were cut, each in seven sections of 0-2.5, 2.5-5, 5-10, 10-20, 20-30, 30-40, 40-50 cm and analysed. Prior to analysis the weight of the soil was recorded and moisture estimated in a Mettler LJ16 Moisture Analyser. Calculations were based on the dry weight of the soil. Radiocarbon in each section was estimated by combustion of 3x500 mg dry samples. Soil sections with radioactivity were extracted with hexane and analysed for amitraz. For extraction, 50 g soil samples were shaken in 200 mL hexane in a horizontal shaker for 4 h, followed by extraction for 8 h with methanol in Soxhlet apparatus. Combined extracts were concentrated in a rotoevaporator at 40°C to dryness, redissolved in 1 mL acetonitrile and analysed by polyamide TLC plates using hexane+acetone (8+2 and 6+ 4, v/v) mixtures.

2.5. Dissipation of amitraz in a field dipping vat

Studies with Amitraz in a commercial cattle dipping vat were performed during July 1991 at a farm in São Paulo State. The vat was filled with water from a pond and treated with Triatox, a commercial formulation of amitraz, to obtain amitraz concentration of 0.42 µg/mL. A total of 360 animals were dipped in the suspension. The procedure used involves an initial dip of the first 30 animals to have the amitraz well dissolved in the vat, followed by two more dips of these animal. Dipping was repeated every two weeks. Samples of the suspension were collected and analysed for amitraz residues. Duplicate 50 mL samples were extracted with hexane (25 mL) by shaking in an horizontal shaker for 30 minutes. The organic phase was separated, filtered on a bed of Na_2 SO_4 and made to 25 mL with hexane in a volumetric flask. The solution was analysed by a gas chromatograph which was equipped with an FID detector and a column packed with 2% OV 17 on chromosorb W.

3. RESULTS AND DISCUSSIONS

3.1. Effects of pH and temperature on the stability of deltamethrin

Deltamethrin was stable at pH 4,7 and 8 and at a temperature below 35°C. However, it was unstable at pH 9. At this pH it degraded at 28°C and above. After incubation for 28 days at 45°, only 16% of the total radiocarbon extracted from the soil and applied to the TLC plates corresponded with the TLC spot for deltamethrin. Two metabolites were also identified on the TLC plates. One of these had Rf values of 0.42 and constituted 81% of the total

radioactivity applied to the plate, and the second had Rf value of 0.66 and corresponded with 16% of the total radioactivity. These metabolites co-chromatographed with standards for 3-phenoxybenzoic acid and 3-phenoxybenzyl alcohol, respectively. After 42 d of incubation in buffer solution at pH 9.0 deltamethrin completely disappeared.

3.2. Degradation of deltamethrin in water and sediment mixture

Distribution of radioactivity evolved as $^{14}CO_2$ and recovered from the water and sediment mixture after 56 d of standing in the biometer flask is shown in Table I. Overall, the evolution of $^{14}CO_2$ was rather low and a maximum of 2.1% of the applied amount of radioactivity evolved as $^{14}CO_2$ when 800 mg sediment was added to the water treated with ^{14}C-labelled deltamethrin in the biometer flask. With lower amount of sediment correspondingly lower amount of $^{14}CO_2$ evolved, and only 0.4% of the radioactivity corresponded with $^{14}CO_2$ when no sediment was added to the water. The addition of 80 mg sediment to the water had no remarkable influence on deltametrhin degradation. However, when the added amount of sediment was increased to 800 mg, there was a decrease in the amount of radioactivity extracted (aqueous phase+organic phase) from the sediment. The analysis of the organic phase by TLC showed that 80% of the radioactivity in the sediment was deltamethrin, which showed a radioactive spot at Rf of 0.71 on the TLC plate. The main metabolite was 3-phenoxybenzoic acid. Sterilization of the sediment prior to incubation had no influence on deltamethrin metabolism.

Table I. Distribution of percent of the applied amount of radioactivity as evolved $^{14}CO_2$ and in the aqueous and organic phases of the extract.

Treatment	Total radioactivity as $^{14}CO_2$ (cumulative) (% of applied)	Radioactivity in Aqueous phase (% of applied)	Radioactivity in Organic phase (% of applied)
Pond water	0.4	86	14
Water plus sediment (80 mg)	1.6	33	66
Water plus autoclaved sediment (80 mg)	1.4	67	32
Water plus sediment (800 mg)	2.1	39	40

3.3. Dissipation of deltamethrin in a model dip vat

Results on the fate of deltamethrin in a model dip vat under field conditions indicate that after standing for 15 days only 25% of the applied radioactivity remained and it corresponded with deltamethrin. After centrifugation of the suspension, the radioactivity present in the sediment corresponded to 50% of the total radioactivity in the sample. The

extraction of the sediment followed by TLC showed that all the radioactivity was deltamethrin. Similar results were obtained when the suspension was allowed to stand in the vat for 30 days. However, analysis of the samples collected from the vat at 60 days showed a decline in the radioactivity. Analysis of the sample after 75 days showed that 30% of the recovered radioactivity was in the solution and 60% in the sediment. TLC analysis of the radioactivity extracted from the sediment showed the presence of only one radioactive spot at Rf 0.70, it contained 98% of the radioactivity applied to the TLC plate and cochromatographed with deltamethrin.

3.4. Dissipation of amitraz

Results of the study on the leaching of amitraz in the soil columns showed that most of the radioactivity was present in the upper 0-2.5 cm section of the soil column (Table II) and it decreased in the lower sections. There was no radioactivity in the sections below 20 cm, indicating that amitraz stongly adsorbs to the soil and is not likely to leach in to the lower strata of the soil or the ground water. TLC analysis showed that 90% of the radioactivity corresponded with amitraz, indicating that this compound is quite persistent.

Table II. Distribution of radioactivity in different section of the soil column after the application of ^{14}C-amitraz.

Soil section (cm)	Percent of the applied amount of amitraz present at different periods of time (d) after the application			
	0	30	60	120
0-2.5	97	76	63	73
2.5-5.0	3.2	18	32	23
5.0-10	-	3.0	3.0	3.4
10-20	-	0.6	1.4	0.4
20-30	-	-	-	-
30-40	-	-	-	-
40-50	-	-	-	-

The results of the analysis of amitraz in the field dip vat showed that the concentration of the pesticide decreased from an initial concentration of 210 μg/mL to 155 μg/mL after 20 d of standing. At that time a recharge was necessary in order to bring the concentration back to 210 μg/mL.

Data obtained from the analysis of the soil columns showed that both amitraz and deltamethrin have low mobility in soil and the risk of groundwater contamination from the discharge of the used dips in to the soil would be expected to be negligible. Moreover, dips in tropical areas are likely to be exposed to high temperatures of 35 to 45°C and, as this study shows, deltamethrin degrades due to the combined affect of high pH (9.0) and temperature (45°C). However, the dissipation can be controlled by monitoring the concentration in the dip and periodic recharging. Although amitraz is metabolized by *microorganisms such as Pseudomonas* and *Achromonas spp* [4] in our study the compound was found to be quite stable under the local field conditions.

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STUDIES ON THE STABILITY OF ACARICIDES IN PANAMA

I. DISSIPATION, DEGRADATION AND BINDING OF ^{14}C - COUMAPHOS IN MODEL CATTLE-DIPPING VATS WITHOUT RECHARGE

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Abstract

An outdoor experiment was carried out in which the stability of ^{14}C-coumaphos was studied in model cattle dipping vats without recharge. Three vats (35L) were treated with ^{14}C-coumaphos as a 220 mg/L suspension. During the 60 days after charging 9%, 72% and 85% of the originally added ^{14}C -activity was measured in the water layer of the vat with high (7%), low (0,1-3%) and no (0%) sediment, respectively. At 240 days, radioactivity in water decreased to about 10%. However, at this time, 50% of the radioactivity was found in the sediment. Extractable residues from sediment were mainly coumaphos (73%) and chlorferone (22%), while potasan and other compounds were detected in small amount (less than 5%). Non-extractable residues bound to sediment were approximately 8%.

1. INTRODUCTION

Many acaricides have been used in Panama during the past. In most cases treatments included the use of organophosphates or pyrethroids. They were used in sprays or in dip vats for the control of ectoparasites. Asuntol, a 20% flowable liquid formulation of coumaphos {0-(3-chloro-4-methyl-2-oxo-2h-1-benzopyran-7-yl)-0,0-diethyl phosphorothioate}, is approved by the Panamanian authorities for the treatment of cattle in dips.

For efficient ectoparasite control it is important that acaricides be used at the correct concentration and at the right time. The problem of resistance development could be substantially reduced if measures are taken to maintain acaricides in dips at recommended concentrations. This requires a clear knowledge of the behavior of the chemical under field conditions by periodically monitoring and development of procedures to improve stability in the vats. Although chemical techniques have been developed to determine coumaphos concentration in dipping-vat fluids [1,2,3,4,5], there is little information on the use of simplified methods or nuclear techniques for this purpose. Therefore, we undertook this study to evaluate dissipation, degradation and binding of coumaphos in model cattle-dipping vats using ^{14}C-acaricide without recharge.

2. MATERIALS AND METHODS

2.1 Chemicals

Analytical grade coumaphos, chlorferone and potasan were gifts from Bayer AG. Asuntol EC 20% was obtained commercially. ^{14}C-Coumaphos (0.0-diethyl-0-3-chloro-4-methyl-(4-14C)-coumarin-7-yl-phosphorothioate) was obtained from Zinta Isotope Trading Enterprise of the Institute of Isotope, Hungary. The radiochemical purity of the ^{14}C-coumaphos was determined by TLC analysis to be 97%.

2.2. Treatment of vats

Three 50 L (20x20x20 cm) concrete vats were installed in the ground at the experimental field site in Tocumen, Panama and protected from direct sunlight. They were charged with 35 litre water and a mixture of non-labelled and ^{14}C-labelled coumaphos to obtain a suspension of the same concentration as that recommended for use in field dipping vats. The recommendation is to add 1 mL Asuntol liquid (containing 20 %, v/v coumaphos) for each liter of water in the dipping vat. The radiolabelled coumaphos had a specific activity of 157,851 dpm/μg or 11,000 dpm/ml in the final suspension. Vats were sampled and allowed to condition for two weeks. Vats-1 and 2 were then treated with a mixture of soil+sediment+manure from a cattle farm. Vat-1 was treated after the initial sampling with 2.5 kg (7%) of the mixture. To vat-2 a smaller quantity (1-50 g) of soil+sediment+manure was periodically added. Total amount of the air-dried mixture added to this second vat increased to 1 kg (3%) at 114 days. No soil+sediment+manure mixture was added to the vat -3.

2.3. Sampling and analysis

Fluid samples (10 mL) were taken from vats in triplicate at o (1 h), 6, 14, 30, 45, 60, 94, 114, 146, 168, 193 and 240 d after adding ^{14}C-coumaphos. The samples were collected with a serological pipette from a depth of 10 cm at the centre of the vat. All samples were taken immediately after bringing water level to the mark (initial level) by adding distilled water and after stirring well for 30 seconds. Samples from vat-2 were taken after adding soil/sediment. The first sample of each vat was taken before addition of soil/sediment and it represents, therefore, the initial reading (0 d, 100%). The pH of the samples and suspended sediment levels were measured after centrifugation for 10 minutes at 3000 rpm and air drying of samples. At the end of the experiment (240 days) water and solid materials of each vat were carefully separated and analyzed. Radioactivity in the samples was estimated in a Packard Tricarb 1000 liquid scintillation counter (LSC). Aliquots of samples were subjected to direct LSC by mixing 1 mL of sample with 10 mL of Instan-gel II. Sediment samples were air dried before combustion in a Harvey OX-600 Biological Oxidizer. Sample extracts were similarly analysis by LSC. In all radiometric measurements corrections were made for instrument efficiency and quench.

Radioactive degradation products in sample extracts were resolved by analysis on thin layer chromatography (TLC) plated coated with alumina containing fluorescence indicator (UV 254). Sample extracts were spotted along one edge of the plate, and the plate developed in n-hexane+acetone (8+2, v/v) mixture. After development the plates were dried and placed on a x-ray film (Kodak X-OMAT-Ar) for periods of up to 14 days for autoradiography. The area of the gel corresponding to radiolabeled compounds was scraped from the plates, transferred into LSC vials, suspended in LSC cocktail and analysed for radioactivity by LSC. Product characterization was achieved by co-chromatography of the radioactive spots from the sample with those of authentic standards for coumaphos (Rf 0.53), chlorferone (Rf 0.19) and potasan (Rf 0.43). The standards for these three compounds were visualized by viewing plates under UV light (365 nm). For HPLC analysis 1.0 mL sample was diluted with 1 mL acetonitrile in a 10 ml test tube. The sample was shaken vigorously and the tube was centrifuged for 10 minutes at 3000 rpm. Analysis of filtered supernatant was performed on a Supelcosil LC-18 column (25 cm length x 0.4 cm dia). The eluting solvent mixture was methanol+water (6+4, v/v) and the flow rate was 1.3 mL/min. The detector wavelength was set at 320 nm. Under these condition the retention time (RT) for coumaphos was 22.8 min, for potasan 16.4 min and for chlorferone 8.8 min. To quantify sediment-bound coumaphos residues samples of air dried sediment (200 mg) were

collected at the end of the experiment (240 d) and combusted for total activity. Samples (7 g) of the sediment were Soxhlet-extracted with methanol for six hours. Aliquots of methanol extract were analysed by LSC for total extractable radioactivity and by TLC-autoradiography for the identification of degradation products. Samples (200 mg) of air dried sediment remaining after Soxhlet-extraction were combusted for quantification and radiocounting of non-extractable (bound) residues.

3. RESULTS AND DISCUSSION

The data on dissipation and degradation of coumaphos in model dip vats and the affect of sediment on its concentration in the suspension are shown in Tables I - IV. The coumaphos content in the suspension taken before and after stirring and measured directly showed a significant difference, but no difference was observed in coumaphos concentration whether or not the samples taken before and after mixing were centrifuged. The initial coumaphos concentration determined after centrifugation was about 60% of that found by direct measurement. This indicates that coumaphos was not completely dissolved and homogeneously distributed in the water, but due to low solubility (50 ppb at 25 ^{0}C) it was suspended in water. The maintenance of a homogeneous suspension of coumaphos in the dipping vat has been reported to be a major problem [6,7].

At the end of 240 d total radioactivity recovered from the vats was 60% of the initially added activity. Coumaphos concentration in vat-1 decreased significantly after 30 d. This may be because of the presence of large amount of soil+sediment mixture added to the vat. After 60 d, only 8.8% of the initial amount of radioactivity was found in the water layer of this vat (Tables I & II). On the other hand, coumaphos concentration remained high in vats with no or low quantity of added soil+sediment after 60 d and even longer. At this time the concentration of coumaphos in vat-2, was approximately 71.9%, and in vat-3 84.8% of the initial concentration (Table III). However, at 240 d after the start of the test radioactivity in the water in all vats was low ($\leq$10% of the initial concentration).

Desorption studies with the radiocarbon absorbed on sediment revealed that about 10% of the radioactivity was released in water. This corresponds with the low radioactivity in the vat fluid measured at the end of the experiment and suggest the presence of an equilibrium of coumaphos distribution between aqueous and solid phases.

It can not be explained why at 240 d total radioactivity recovered from vat-3, to which no sediment had been added, was less than that measured in the other vats to which sediment had been added. It indicates that dissipation of the acaricide did take place in vat-3, and this may have occurred due to the presence of large populations of microorganisms, including algae which were observed in this vat at 114 d after the treatment.

Table IV shows data on radioactivity in the sediment at 240 days. The radioactivity in the sediment of vats-1 and 2 was 47 - 51%, respectively of the initial concentration. But the concentration in the solids in vat-3, which were primarily from algae, was only 16%, indicating that soil+sediment are much better adsorbents for coumaphos than microorganisms and their residues.

TABLE I. DISSIPATION OF RADIOLABELLED COUMAPHOS IN SUSPENSION SAMPLES FROM DIP NO. 1

PERIOD OF TIME (DAYS) AFTER THE TREATMENT	CONCENTRATION OF REDIOACTIVE COUMAPHOS						
	AB		DB		DB + VOL		pH
	dpm/mL	%	dpm/mL	%	dpm/mL	%	
0 (1hr)	3965	100	3965	100	3965	100	7.50
1 (24hr)	3764	94.9	3800	95.8	3764	94.9	7.71
14	2128	53.7	3443	86.8	2956	74.6	8.00
30	1093	27.6	2096	52.9	3000	75.7	8.30
60	349	8.8	415	10.5	365	9.2	8.60
81	264	6.7	464	11.7	486	12.3	8.62
94	251	6.3	525	13.2	426	10.7	8.70
111	253	6.4	475	12.0	400	10.1	8.80

AB : SAMPLING WITH NO STIRRING
DB : SAMPLING AFTER STIRRING
DB+VOL: SAMPLING AFTER ADDING WATER AND STIRRING

TABLE II. CONCENTRATION OF RADIOLABELLED COUMAPHOS IN SAMPLES FROM DIP NO. 1 TAKEN BEFORE AND AFTER STIRRING AND CENTRIFUGED.

PERIOD OF TIME (DAYS) AFTER THE TREATMENT	CONCENTRATION OF RADIOLABELLED COUMAPHOS						
	AB dpm/mL	%	DB dpm/mL	%	DB + VOL dpm/mL	%	pH
0 (1hr)	2050	51.7	2050	57.7	2050	57 7	7 50
1 (24hr)	1940	48.9	1940	48 9	1940	48.9	7 71
14	1700	42.9	1609	40.6	1440	36 3	8 00
30	838	21.1	869	21.9	932	23.5	8 30
60	348	8.8	414	10.4	365	9 2	8 60
81	225	5.7	346	8.7	230	5 8	8 62
94	220	5.6	250	6 3	231	5 8	8 70
111	254	6.4	365	9.2	260	6.6	8 80

AB : SAMPLING WITH NO STIRRING
DB . SAMPLING AFTER STIRRING
DB+VOL. SAMPLING AFTER ADDING WATER AND STIRRING

TABLE III. DISSIPATION OF RADIOLABELLED COUMAPHOS IN DIPS TREATED WITH AND WITHOUT ADDED SEDIMENT.

PERIOD OF TIME (DAYS) AFTER THE TREATMENT	CONCENTRATION OF RADIOLABELLED COUMAPHOS					
	VAT #2			VAT #3		
	dpm/mL	%	pH	dpm/mL	%	pH
0	11020	100.0	8 10	10918	100 0	7.50
(1h)	10878	98.7	8 13	10918	100 0	8 11
6	10514	95.4	7.26	10900	99.8	8 13
14	9272	84.1	8.29	10832	99.2	8.10
31	8778	79 7	7 63	10976	100 5	8 25
45	7794	70.7	8.20	10288	94 2	7 70
60	7920	71 9	8 23	9260	84 8	7 85
86	2968	26.9	7.65	9842	90 1	8 25
114	2792	25 3	7.65	8014	73 4	8 15
146	1608	14.6	8.30	5166	47.3	8.33
168	1546	14 0	8.17	6796	43 9	8 68
193	924	8.4	8 25	1912	17.5	8 55
240	974	8.8	8.45	930	8 4	8 50

DIRECT LSC - MEASUREMENTS.

TABLE IV. DISTRIBUTION OF COUMAPHOS RESIDUES BETWEEN LIQUID AND SEDIMENT IN DIPS AFTER 240 DAYS OF THE TEST.

VAT #	AMOUNT OF SOLID (kg)	DPM IN FLUID (%)	DPM ON SOLID (%)	RECOVERY (%)
1	2.5	9 020 250 (13.8)	31 237 500 (47.4)	61.2
2	1.0	35 269 500 (9.1)	197 472 633 (51.2)	60.3
3	0.009 (algae)	32 690 000 (8.8)	61 235 325 (16.3)	25.1

At the end of the experiment 92.4% of the radiocarbon in the sediment was extracted with hot methanol and the remaining 7.6% was non-extractable. HPLC analysis of the extractable residues showed that it contained primarily the parent compound coumaphos (72.8%) and the degradation product chlorferone (22.4%). Small quantities (≤5%) of potasan and unidentified polar metabolites were also found by TLC-autoradiography/LSC. The pH of the vat suspension at the start of the experiment was slightly basic (pH 7.5), but later it increased, and after 30 days reached pH 8.3. Later still it increased further to pH 8.8. The study also indicates that volatility was not a major factor for the loss of the acaricide from the vats, and less than 2% coumaphos was lost due to volatility. Adsorption on the walls of vats was limited to 2% of total radioactivity.

4. CONCLUSIONS

1. Coumaphos concentrations in vat fluids gradually decreased with time.
2. Total radioactivity recovered from the vats toward the end of the experiment was about 75% of the radioactivity initially added.
3. Radioactivity in the vat fluid was low after 6 months, regardless of the presence or absence of sediment in the vat.
4. The half life of coumaphos was 6-10 week in vats with sediment and 20 weeks in vat without sediment.
5. Residues mainly consisted of coumaphos in the sediment and degradation product were mostly chlorferone and potasan.
6. The pH in dipping vats was initially basic and increased to 8.8 during the period of study.
7. Little loss occurred by volatilization or adsorption on the walls of the vats.

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II. THE EFFECT OF MICROBIAL ACTIVITY ON THE STABILITY OF ^{14}C-COUMAPHOS

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Abstract

The effects of sediment adsorption and microbial activity on the stability of ^{14}C-coumaphos in a water+sediment system was studied by quantifying mineralization, distribution between water and sediment phases and binding to sediment Mineralization of Coumaphos slowly increased with time but it decreased slightly with an increase in sediment content. Incubation of non-sterilized and sterilized water+sediment systems treated with ^{14}C-coumaphos for 12 weeks resulted in losses up to 14% in non-sterile treatments. Most of the added radioactivity was found in the sediment. The extractable residues (60%) contained mainly coumaphos and chlorferone.

1. INTRODUCTION

The stability of acaricides in the environment is influenced by biological factors such as microorganism and nonbiological factors. Considerable research has been conducted on the environmental properties of Coumaphos. It has been reported that microbial degradation of coumaphos in vats occured causing loss of efficiency. Degradation rates were variables. The half-life of coumaphos in aqueous solution was 23 d at pH 9.0 (50 °C) and less that 7 d at pH 5.5 (20-35 °C). Further, it is reported that in sterile aqueous solution of coumaphos less than 10% degradation was observed after 30 days incubation at pH 5 and pH 7, whereas approximately 16% degradation by hydrolysis was observed at pH 9 under same condition [1,2,3,4,5].

In a model experiment conducted outdoors at our Laboratory we found after 8 months exposure in vats about 40% loss of the radioactivity added as ^{14}C-coumaphos.

The purpose of this research was to evaluate the role played by the sediment/manure (high or low) and microbial activity on degradation and binding of ^{14}C-coumaphos in biometer flasks containing soil+water system under laboratory conditions. Further objective of the study was to provide supporting data to the field and model studies on stability of coumaphos in dipping vats.

2. MATERIALS AND METHODS

2.1. Chemicals

Analytical grade coumaphos, chlorferone and potasan were gifts from BAYER AG. ^{14}C-coumaphos (0,0-diethyl-0-1/3-chloro-4-methyl-(4-^{14}C)-cumarin-7-yl-phosphorotioate) was obtained from Zinta Isotope Trading Enterprice of the Institute of Isotope-Hungary. The radiochemical purity of the ^{14}C-coumaphos was determined by TLC studies to be 97%. Ascarite II, 30-40 mesh, anhydrous Na2SO4, KOH and analytical grade solvents were used.

2.2 Experimental set-up

The experiment was conducted using four sets of biometers in triplicate charged with 50 mL river water and 50 µL preparation of commercial Asuntol 20% to get a final concentration of 200 mg/L [6]. Two sets of the biometer flasks were treated with 0.3 g of air dried soil/sediment (low sediment, 0.6%) from a cattle farm in Parita. Two other sets of biometers were treated with 3 g of the same soil/sediment (high sediment, 6%). To the sidearm of each biometer flask 10 ml of 0.1M KOH was added. To each of six 250 mL biometer flask from both sterile and non-sterile sets 65,403 dpm ^{14}C-coumaphos was added The specific activity of the final preparation was 157,851 dpm/µg coumaphos. Incubation was continued at ambient temperature (23-26 °C) in the laboratory for 12 weeks.

2.3 Sampling and analysis

Samples (0.5mL) of 0.1M KOH solution were removed in duplicate weekly from biometer flask and counted by mixing with 10 ml Instan-gel II in a liquid scintillation counter (LSC) Packard Model 1000. Biometers were recharged with 0.5 mL of 0.1M KOH solution. At the end of experiment (89 d) biometer flasks were dismantled, water and solid matter in each flask carefully separated and ^{14}C-activity determined. Sediment samples were air dried before oxygen combustion on a Harvey Biological Oxidizer OX-600. In all radiometric measurements appropiate corrections were made for instruments efficency and quench.

3. RESULTS AND DISCUSSION

The data on radiocarbon trapped in KOH solution and radioactivity distribution in the biometer system as determined by procedures described above are presented in Tables I, II and III. The 14C-activity released in suspension remained low but it increased smoothly with time up to 14%. The ^{14}C released in non-sterile flasks with high content of sediment was higher than in sterile flasks. However, this was not observed in flasks with low soil/sediment content.

Toward the end of the experiment degradation of total originally added ^{14}C-coumaphos was in the range of 12-14% in flasks low in sediment addition and 6-8% in flasks with a high sediment level.

After 3 months of incubation large quantities of the 14C-activity were measured in sediment (up to 80%). In water layer 24-60% of the originally added radioactivity was found. At this time pH was in the range 8.3 - 8.5.

At the end of the experiment about 60% of radiocarbon in the sediment was extracted with acetonitrile. The extractable residues as identified by TLC-autoradiography were mainly coumaphos and chlorferon.

4. CONCLUSIONS

1. The released 14C-activity from 14C-coumaphos suspension was low and increased with time up to 14% after 12 weeks.

TABLE I. RADIOACTIVITY RELEASED WITH TIME AS $^{14}CO_2$ FROM COUMAPHOS IN BIOMETER FLASKS.

days since charge	0.3g Sediment dpm total	%	3.0g Sediment dpm total	%	0.3g Sediment dpm total	%	3.0g Sediment dpm total	%
0	0	0	0	0	0	0	0	0
5	6660	1.0	4620	0.7	5980	0.9	2310	0 4
8	10603	1.6	6260	1.0	16970	2.6	5470	0.8
16	23894	3.7	21876	3.3	23727	3.6	11823	1 8
23	31366	4.8	26307	4.0	29733	4.5	18424	2 8
33	36687	5.6	31648	4.8	42792	6.5	21104	3.2
37	44115	6.7	36136	5.5	46134	7.1	26986	4 1
50	56396	8.6	38177	5.8	49366	7.5	28874	4.4
56	65749	10.1	45240	6.9	64159	9.8	33961	5.2
64	74300	11.4	38440	5.9	69204	10.6	36962	5.6
77	62260	9.5	40998	6.3	82108	12.6	50554	7.7
89	79600	12.2	39043	6.0	91955	14.0	50696	7.8

TABLE II. DISTRIBUTION OF RADIOACTIVITY BETWEEN WATER AND SEDIMENT IN THE BIOMETER FLASKS AFTER 89 DAYS OF INCUBATION.

BIOMETER SYSTEM	14C02		14C IN WATER		14C ON SEDIMENT		RECOVERY (TOTAL 14C)	
	dpm	%	dpm	%	dpm	%	dpm	%
N S + 0.3g Sed	79600	12.2	358715	54.8	222274	33 0	660589	101
N S + 3.0g Sed	39043	6 0	153822	23.5	515295	70 5	631792	98 5
S + 0 3g Sed	91966	14 0	380280	58.1	229370	27 9	701616	107 3
S + 3 0g Sed	50696	7 8	158671	24.2	509417	68 0	639351	99 4

N.S. Non-sterilized sediment
S Sterilized sediment

TABLE III. DISTRIBUTION OF EXTRACTABLE AND SEDIMENT-BOUND RESIDUES IN BIOMETER FLASKS AFTER 89 DAYS OF INCUBATION.

TRATMENT	EX-TRACTABLE DPM	%	BOUND DPM	%	TOTAL %
non Sterile + 3g Sediment	303 464	46.4 (58.4%)	209.286	32.4 (47.6%)	78.8 (100%)
Sterile + 3g Sediment	284 904	43.6 (56%)	223.480	34.2 (46%)	77.8 (100%)

2. Non-steril Biometer system high in sediment content released more 14C-activity than steril system.
3. After 12 weeks, degradation of 14C-Coumaphos was 12-14% for biometer low in sediment and 6-8% for biometer with a high sediment level.
4. After 3 month incubation the highest amount of ^{14}C-residue was measured on sediment, but considerable levels of ^{14}C-activity was found in water layer (24-60%).
5. Residues on sediment were mainly coumaphos and chlorferon.

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III. A STUDY OF THE AFFECT OF TEMPERATURE, pH AND THE ADDITION OF SEDIMENT AND BUFFERING AGENTS ON THE STABILITY OF COUMAPHOS AND DELTAMETHRIN UNDER LABORATORY CONDITIONS

(Abstract)

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The affect of temperature, pH, and the addition of buffering agents, salts and lime on the stability of coumaphos (Asuntol 20%) and deltamethrin (Butox 6%) in the presence or absence of sediment was studied in glass bottles under laboratory conditions. The salts included ammonium acetate, sodium carbonate and sodium chloride and the buffering agents were superphosphate fertilizer and phosphate buffer (pH 7.2) containing potassium dihydrogen phosphate and potassium hydroxide. The concentration of the acaricides was monitored by using HPLC. Samples were taken at 0, 7, 14, 21, 30 and 60 d and analysed for the parent compounds by HPLC using a Supelcosil C-18 column and acetonitrile as eluting solvent. There was some affect of temperature on the stability of coumaphos and 76% of the original amount of coumaphos was present after 60 d at 10°C and 64% at 25°C. The suspension treated with lime had the minimum concentration of the acaricide and only 2 and 3% of the acaricide remained in the presence and absence, respectively, of the sediment, indicating its instability highly basic conditions (pH 13). The concentration was also reduced by the addition of sediment to the suspension. It was more so when the salts were also present. At 25°C the acaricide was most stable in phosphate buffer (pH 7.2) and 78% of the original amount was present after 60 d, whereas, 43% was present in the suspension to which superphosphate fertilizer (pH 3.4) had been added. After 60 d 62-63% of deltamethrin remained in the aqueous solution at pH 6. The recovery was lower in phosphate buffer (54%) and the addition of superphosphate (20%). No deltamethrin was detectable in containers to which lime was added (pH 13).

IV. STUDY OF THE STABILITY OF ^{14}C-LABELLED COUMAPHOS IN THE PRESENCE OF SEDIMENT, AN ACIDIC BUFFER AND A BACTERIOSTATIC AGENT IN MODEL DIPPING VATS UNDER OUTDOOR CONDITIONS

(Abstract)

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The affect of the addition of sediment, a buffering agent (superphosphate fertilizer) and a bacteriostat (copper sulphate) on the stability of ^{14}C-labelled coumaphos in suspension in model dipping vats was studied for a period of 180 days under outdoor conditions. At the end of this period the highest proportion of the initial radioactivity (36%) was in the vats containing coumaphos suspension with no additives and the lowest (10%) in the vats in which the bacteriostat and sediment were added to coumaphos suspension. Recovery was lowered in all vats to which sediment or the bacteriostat had been added. In the presence of sediment or the bacteriostat addition of the buffering agent did not have any affect on the levels of radioactivity after 180 days. Thus, the addition of superphosphate to coumaphos suspension containing bacteriostat reduced the radioactivity from 26% to 23% of the initial amount. The radioactivity level was 10% in the suspension to which sediment had been added and 12 % to which both sediment and superphosphate were added.

V. MONITORING THE STABILITY OF COUMAPHOS ACARICIDE IN FIELD CATTLE DIPPING VATS BY USING HPLC

(Abstract)

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The concentration of coumaphos in four field cattle dipping vats and its distribution at different depths in the suspension and in the sediment was monitored over 12 to 48 weeks. The residual concentration of coumaphos and degradation products was determined by HPLC. Coumaphos was extracted from the suspension by shaking with equal volume of methanol and 90% of coumaphos was extracted by this method. A reverse phase C-18 column (25 cm x 0.4 cm) was used in the HPLC and the eluent was a mixture of methanol+ water (80+20,v/v). The initial concentration of coumaphos was 200 mg/L. However, it steadily decreased in all four vats with time. The final concentration was reduced to 39% of the concentration at zero time in vat # 1 after 12 weeks, 17% after 18 weeks in vat # 2, 29% after 19 weeks in vat # 3 and 23 % after 48 weeks in vat # 4. The concentration in the sediment increased from 165 mg/kg at zero time to 1960 mg/kg after 18 weeks in vat # 1 and 152 mg/kg to 2020 mg/kg after 48 weeks in vat # 4. The concentration of coumaphos in the suspension ranged between 28 to 81 mg/L at the surface, 46 to 115 mg/L at 20 cm and 86 to 147 mg/L at 100 cm depth.

VI. A STUDY OF LEACHING OF ^{14}C-LABELLED COUMAPHOS IN SOIL COLUMNS

(Abstract)

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Leaching of ^{14}C-labelled coumaphos from aged dipping vat suspension was studied in 50 cm long x 20 cm diameter cores of undisturbed soil in mini-lysimeters. The vat suspension, previously aged for 6 months, was deposited to the top of the columns in lysimeters which were kept outdoors. The leachate was periodically collected over 6 months and analysed for radioactivity. At the end of this period soil columns were cut in 10 cm sections and the radioactivity present in each section was determined by combustion of homogenized soil samples. Radioactivity was also extracted from the soil samples with methanol by using Soxhlet apparatus and the extract analysed for total radioactivity. The extract was analysed by TLC for coumaphos and degradation products. No radioactivity was detected in the leachate. Most of the radioactivity (91-100%) was found in the top 10 cm section of the soil, indicating that coumaphos in the discarded waste does not leach below the top section of the soil and does not have high potential to contaminate ground water. Of the total radioactivity in the top section 62 -75% was extractable and rest remained soil bound. The extractable radioactivity was coumaphos and no degradation products were detected.

LEACHING BEHAVIOR OF COUMAPHOS FROM A SPENT CATTLE DIP AND ITS BIOAVAILABILITY TO PLANTS

(Abstract)

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The leaching behavior of coumaphos and its degradation products from a simulated cattle dip suspension was studied one year after exposure to field conditions. The bioavailability of soil-bound coumaphos to cucumber and sedge was also determined using radiotracer techniques.

A major portion of the spent dip suspension (41%) was bound to soil in the top 10 cm. Although coumaphos was found to be immobile on soil TLC plates, mass flow and the presence of water-soluble metabolites resulted in minimal leaching of the spent dip suspension. Disposal by draining of the spent dip suspension does not poze a hazard for ground water contamination.

Uptake of very low level of soil-bound residues, which correspond to 0.003 - 0.02% of the applied acaricide, was observed. The levels found in plants were determined to be of no toxicological importance to cattle which may feed on plants growing in cattle dip waste disposal areas.

CATTLE DIPPING PRACTICES IN THE PHILIPPINES AND THE DEGRADATION OF COUMAPHOS IN A SIMULATED CATTLE DIP

(Abstract)

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A survey of cattle dip facilities and current practices employed was done. Coumaphos and ethion were the commonly used acaricides in the four respondent stock farms. The behavior of coumaphos in a simulated model cattle dip was monitored using radiotracer techniques. Degradation was rapid, resulting in the formation of potasan metabolite and bound residues in the sediment.

A rapid field method for the detection of organophosphate pesticides was used in monitoring the degradation of coumaphos in a cattle dip. The sensitivity of the method is comparable to the conventional HPLC method employed. This rapid field method can easily be used by cattle ranch owners to monitor coumaphos content of the vat facility so that recharging could be made in order to prevent the onset of resistance development in cattle tick.

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