UPTAKE OF KEPONE® BY AQUATIC MACROPHYTES

by

Kenneth A. Langeland

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APPROVED:

S. W. Bingham, Co-Chairman

J. A. Swader, Co-Chairman

M. G. Hale

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^{14}C-Kepone and technical Kepone were supplied by Allied Chemical Company.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

Kepone®(decachloro-octahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalen-2-one) is in the cyclodiene family of insecticides. Since the trade name has come into more general usage than the common name, chlordecone, the chemical will be referred to as Kepone throughout this paper.

Kepone is synthesized by the dimerization of hexachlorocyclopentadiene in the presence of sulfur trioxide, followed by hydrolysis to the ketone (Martin & Worthington, 1974). The process results in a highly stable six membered cage structure which, with the exception of the keto-oxygen, is chlorinated at each apex (Figure I-1). The anhydrous Kepone molecule readily hydrates when in contact with normal atmospheric humidities forming a monohydrate, dihydrate, and trihydrate. The geminal diol, thus formed, is very polar, accounting for the higher water solubility (1.5 to 2.0 $mg \cdot l^{-1}$ at pH 4 to 6, 5.0 to 70 $mg \cdot .^{-1}$ at pH 9 to 10) than other organochlorine compounds (Martin & Worthington, 1974). The molecule also forms addition products at the keto-oxygen with alcohols, amines (McBee et al., 1956; Gilbert et al., 1966a, 1966b) and ketones (Gilbert et al., 1966a).

Kepone was introduced in 1952 by Allied Chemical Company. Although domestic use was limited to small quantities

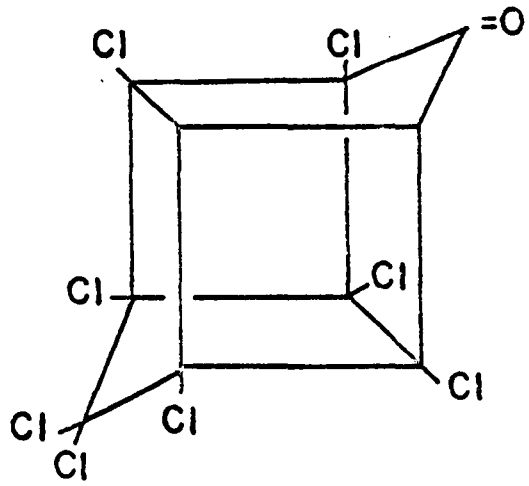

Figure I-1. Anhydrous Kepone

used in ant and roach baits, large quantities were produced in the United States and transported to Central America and Europe. Approximately 1.5×10^6 kg were produced in Hopewell, Virginia between 1966 and 1975 (USEPA, 1978a).

Kepone, which was little known to the American public before 1975, became a subject of widespread public concern when an investigation by Virginia State Public Health Officials, in July 1975, revealed Kepone poisoning of personnel at the Life Sciences Products Company. In response to this discovery and evidence of local environmental contamination, the company, which was producing Kepone under contract to Allied Chemical Company, closed voluntarily.

Subsequent investigations by state and local agencies have revealed Kepone contamination of approximately 118 km of the James River between Hopewell, Virginia and Newport News, Virginia. Current estimates indicate that 9,000 to 17,000 kg of Kepone are present in the top 30.5 cm of river sediment (USEPA, 1978a). The magnitude of this environmental contamination has led to fishing bans, restrictions on shellfish harvesting, economic losses, and legal issues.

The environmental impact of Kepone contamination in the James River is not presently understood, and is under active investigation. Although Walsh et al. (1977) found the toxicity of Kepone to four algal species to be from 0.35 to 0.60 µg/g (dry weight or fresh weight not indicated), phytoplankton populations in the James River contain an average

of 1.30 µg/g (dry weight or fresh weight not indicated) (USEPA, 1978b). It is, therefore, uncertain at this time whether phytoplankton populations are adversely affected in the James River. It is clear, however, that phytoplankton do present a source of Kepone at the base of the food chain. Bahner et al. (1977) showed that the green alga, Chlorococcum sp., bio-concentrated Kepone from water in laboratory bioassays. When fed Chlorococcum containing approximately 34 µg Kepone/g wet weight, oysters attained 0.21 µg Kepone/g (wet tissue) in 14 days.

Kepone accumulation by higher plants has not been studied in detail. Investigations at the Batelle Memorial Institute showed that Kepone was not taken up by barley seedlings (USEPA, 1978b). It is important to note, however, that Mirex^{R}, which has been shown to photo-degrade to Kepone (Carlson et al., 1976), was shown to be taken up by bean and pea roots, and to be translocated to aerial plant parts (Mehendale et al., 1972).

Several aquatic plants have been shown to remove toxic organic compounds (Seidel, 1966; Wolverton, 1976) and pesticides (Bingham, 1973) from water. It is therefore important to investigate the accumulation of Kepone by aquatic macrophytes which occur in the James River in order to determine if the chemical is thereby made available for transfer to primary consumers. The following studies were undertaken

to determine if Kepone is taken up and translocated by aquatic macrophytes, and to seek evidence for metabolism of the Kepone molecule.

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II. UPTAKE AND TRANSLOCATION OF KEPONE® IN AQUATIC PLANTS

Abstract

Arrowarum [Peltrandra virginica (L.) Schott & Endl.], waterhyacinth [Eichhornia crassipes (Mrt.) Solms], American bulrush [Scirpus americanus (Pers.)], and smooth cordgrass [Spartina alterniflora (Loisel.)] removed Kepone® (decachloro-octahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalen-2-one) from water. ^{14}C-Kepone was translocated from roots and accumulated in small, but significant, quantities in aerial portions of the plants. Approximately 80% of the ^{14}C-Kepone was removed from solution within three days by arrowarum and waterhyacinth; however, very little additional Kepone was accumulated by these species after the initial three day exposure. Kepone uptake by the roots of American bulrush and smooth cordgrass appeared to continue for a seven day period. Translocation to aerial portions of the plants was slow, with significant quantities reaching the shoot after seven days.

Introduction

Aquatic vegetation has been shown to remove organic chemicals from natural and waste water by Seidel (1966), who demonstrated the removal of a wide variety of organic

compounds by giant bulrush [Scirpus validus Vahl.] and Wolverton (1976), who demonstrated that waterhyacinth [Eichhornia crassipes (Mart.) Solms] removed phenol from water. Emersed aquatic macrophytes were also shown effective in removing certain pesticides from water by Bingham (1973).

Kepone accumulation by higher plants and algae has been investigated to some extent. Bahner et al. (1977) found that the green alga, Chlorococcum sp., accumulated large amounts of Kepone. Walsh (1977) also demonstrated Kepone accumulation by Chlorococcum sp., Dunaliella tertiolecta, Nitsachia sp. and Thalassiosira pseudonana. In contrast, studies at the Battelle Memorial Institute and Virginia Institute of Marine Sciences (USEPA, 1978) have not yielded evidence of Kepone accumulation by barley seedlings or rooted aquatic plants.

The closely related compound, Mirex® (decachloro-octahydro-1,3,4,-metheno-2H-cyclobuta [cd] pentalen), that photodegrades to Kepone in the environment (Carlson et al., 1976) has been studied in more detail. Mirex is accumulated by Johnsongrass, annual ryegrass, tall fescue, alfalfa, alsike clover, and other crop seedlings, in direct proportion to the concentrations in the rooting media (de La Cruz, 1975). Pea and bean seedlings accumulated Mirex in their roots to concentrations of six to ten times that amount contained in contaminated water (Mehendale, 1972).

The objective of the present study was to determine the extent of ^{14}C-Kepone uptake from water by selected aquatic macrophytes, and distribution to various parts of these plants.

Selection of plant species to be investigated for Kepone accumulation was based on two criteria: (1) occurrence in, or adjacent to, Kepone contaminated areas and (2) utilization by wildlife. The first criterion was determined by making several field investigations of the littoral and sublittoral zones of the James River which are contaminated with Kepone. These areas included Bailey Creek, Bailey Bay, and the confluence of the James and Chickahominy Rivers. Inspection of submersed vegetation was accomplished by visual observations and sampling with a dredge.

Investigation of the Kepone contaminated areas of the James River revealed the following emergent hydrophytes to be in abundance at the time of observation:

Spartina alterniflora Loisel.

Spartina cynosuroides (L.) Roth

Zizania aquatica L.

Scirpus americanus Pers.

Scirpus robustus Pursh.

Scirpus validus Vahl.

Sagittaria latifolia Willd.

Peltandra virginica (L.) Schott and Endl.

Pontederia cordata L.

From this group of important plants, three were selected for study: arrowarum [Peltandra virginica (L.) Schott and Endl.], American bulrush [Scirpus americanus (Pers.)], and smooth cordgrass [Spartina alterniflora (Loisel.)]. Eventually, all of these species should be investigated for Kepone accumulation. This list is included as a recommendation for further study.

An incentive for studying the accumulation of Kepone by plant species that occur in the area contaminated with Kepone is to contribute information concerning the availability of the chemical for food chain transfer in the James River ecosystem. Thus, the second criterion was satisfied by selection of plant species with documented wildlife utilization. The seeds of arrowarum are consumed by waterfowl and muskrats (Fassett, 1957; Silberhorn, 1976). The seeds of smooth cordgrass are consumed by waterfowl and songbirds (Correll, 1972) and the rhizomes are a favorite food of geese (Silberhorn, 1976). Muskrats are known to feed on the underground plant parts of Spartina sp. and to use the stems and foliage in lodge construction. The extreme importance of smooth cordgrass in the detrital food chain of estuarine ecosystems is well documented (Odum, 1971). The seeds of American bulrush are consumed by waterfowl, and

marshes abundant in this species are considered to support the most muskrats in tidewater Virginia (Silberhorn, 1976).

Waterhyacinth [Eichhornia crassipes (Mart.) Solms] does not occur in the James River area but was also included. Waterhyacinth was chosen for this study because removal of organic chemicals, from water, by these plants has been demonstrated (Wolverton, 1976). Since the plants are free floating in habit and can be harvested with relative ease, they may be useful in efforts to mitigate Kepone contamination of the James River.

Materials and Methods

Propagation

American bulrush and smooth cordgrass rhizomes, and arrowarum seeds were collected during September and October, 1977 along the east and west shores of the Chickahominy River, just north of the S. R. 5 bridge, and on tidal flats to the east of the main river channel approximately 0.4 km north of the bridge.

Rhizomes were thoroughly rinsed of river sediment and cut into 8.0 to 12 cm sections which were then placed 4.0 cm deep in plastic pots containing sand and peat (1:1). The pots were placed on greenhouse benches which were lined with polyethylene film and filled with 0.25 strength Hoagland's solution (Hoagland & Arnon, 1950). Cutrine® was

added to the nutrient solution, at a rate of 0.5 $mg \cdot l^{-1}$ copper equivalent, to reduce algal growth.

Arrowarum seeds were washed with tap water, surface sterilized with a 0.5% NaOCl solution for one minute, and stored in glass jars at 12 C. After a minimum of one month cold treatment, the seeds were germinated in plastic pots containing sand and peat (1:1) and maintained on greenhouse benches as previously described.

Waterhyacinth plants were obtained from existing greenhouse stock and maintained on greenhouse benches as described above.

Treatment Solutions

A ^{14}C-Kepone (s. a. 35.71 μCi/mg) stock solution was prepared by dissolving 4.0 mg in acetone. To determine if clean-up procedures were necessary, 2.0 μl of the stock solution was spotted on a thin layer chromatography (TLC) plate (200 μm silica gel) and developed with methanol/chloroform (1:9 v/v). X-ray film was then used to reveal the locations of ^{14}C-compounds on TLC plates. Radioactivity remaining at the origin, at Rf 0.05, and at Rf 0.66 was scraped separately from the plate and counted in 15 ml Aquasol® with a Beckman LS-250 liquid scintillation counter. The procedure was repeated three times and percent of the total radioactivity in each spot calculated to be 2.8% at the origin, 0.8% at RF 0.05, and 96.4% at Rf 0.66. The solvent was

then evaporated and the ^{14}C-Kepone redissolved in 0.05 N NaOH which was the form added to the nutrient solution for plant treatments.

A stock solution of technical Kepone (89% pure as determined by gas liquid chromatography) was prepared by dissolving 41.7 mg in 100 ml of 0.05 N NaOH.

The treatment solution consisted of Gerloff's medium (Gerloff, 1973), pH 7.5 prepared with glass distilled water, ^{14}C-Kepone, and technical Kepone. Total Kepone concentration was 1.83 µM. Radioactivity in the solution was determined by liquid scintillation counting (LSC).

Exposure of Plants to ^{14}C-Kepone

After washing the roots of arrowarum, American bulrush, waterhyacinth, and smooth cordgrass with a fine mist of tap water, twenty-four plants of each species were placed in an aquarium with their roots submersed in Gerloff's medium, pH 7.5, overnight. The plants were then placed in glass jars (one plant per jar) containing the previously described treatment solution, and the jars wrapped with aluminum foil. To keep the shoots upright, and prevent them from coming into contact with the treatment solution, each plant was taped lightly to a wooden stake attached to the jar. The top of each jar was sealed with aluminum foil, and non-absorbant cotton was placed between the foil and shoot. Jars containing treatment solution but no plants were sealed

with aluminum foil to determine if Kepone was lost by adsorbance to the glass or by volatilization.

The plants were incubated in this way, on a greenhouse bench, for three, five, and seven day periods from the time they were placed in the treatment solution. At the end of each of these incubation periods, three plants were selected at random and removed from the treatment solution for radioassay.

The studies were conducted between February and May, 1978. Average day and night temperatures were 29 and 16 C, respectively. Average relative humidity was 60%.

Quantification of Radioactivity

Plants were removed from the treatment solution and the roots were rinsed for five minutes under running tap water to remove excess treatment solution and unbound ^{14}C-Kepone. The roots were blotted dry, roots and shoots separated (rhizomes included with roots), weighed, placed in paper bags, and oven dried at 45 C for three days. After drying, the tissue was weighed, and ground with a Wiley mill to pass through a 40 mesh screen. Three sub-samples from each shoot and root were placed into pre-weighed combustible polycarbonate capsules, capped and weighed to determine sub-sample weight. Plants from untreated nutrient solution were prepared in the same manner.

Radioactivity in shoots and roots was determined by combusting the tissue with an Intertechnique IN-4101 L. S. Sample Oxidizer, and counting the collected $^{14}CO_2$ with a Beckman LS-250 liquid scintillation counter.

The oxidizer was checked for efficiency by micropipetting a sample of ^{14}C-Kepone solution, of known radioactivity, into a capsule containing untreated plant material, combusting, and calculating the percentage of the known radioactivity recovered. This process was repeated three times each time the oxidizer was used, and the efficiency found, consistently, to be 98%. All combustion data was corrected for background and efficiency.

The treatment solution remaining in the jars was measured, and the radioactivity was determined by counting three one-ml aliquots from each jar.

Distribution of Radioactivity in Plants

Plants were autoradiographed similar to the methods of Crafts and Yamaguchi (1964). After removal from the treatment solution, the roots were washed for five minutes under running tap water, roots and shoots separated, mounted on non-absorbant paper, and oven dried at 45 C for three to five days. After oven drying, the plants were pressed overnight between sheets of foam rubber, backed with aluminum. The plants were then autoradiographed with

medical X-ray film. Roots were autoradiographed for three days and shoots for five weeks. Plants from untreated nutrient solution were autoradiographed in the same manner as checks.

Results and Discussion

Removal of Kepone from Water

The radioactivity of treatment solutions which contained no plants ('0' days of treatment) remained unchanged during the study (Figure II-1). This is consistent with other data which show that Kepone is not lost from solution by volatilization (USEPA, 1978). Decreases in the radioactivity of the nutrient solutions were significantly different by the least significant range procedure (LSR) for all species investigated in the three, five, and seven day incubations. However, there was no significant difference among these incubation periods.

The decreases in Kepone concentration observed between the initial and longest incubation periods were arrowarum, 82%; American bulrush, 60%; smooth cordgrass, 63%; and, waterhyacinth, 77%. The data indicate that the rooted plants may remove substantial amounts of Kepone from hydrosoil. However, the adsorptive properties of organic river sediment may overcome this effect to some extent. The reduction of Kepone concentrations in treatment solutions

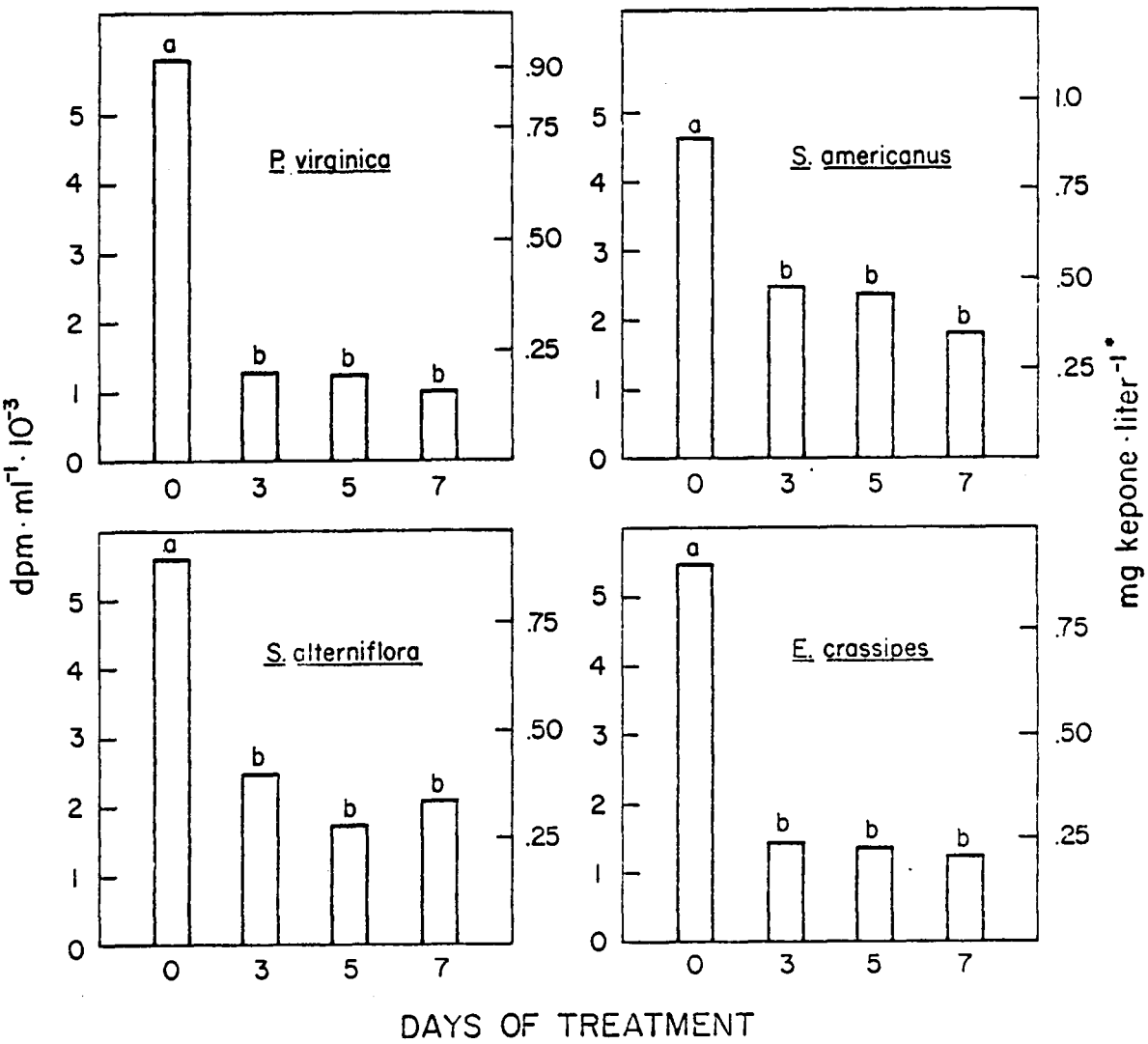


Figure II-1. Radioactivity and Kepone concentration remaining in solution after treatment of arrowarum (P. virginica), American bulrush (S. americanus), smooth cordgrass (S. alterniflora), and waterhyacinth (E. crassipes) and in treatment solution containing no plants (0-days). Each bar represents an average of three replications. Bars in individual graphs with the same letter are not significantly different at the .05 level of significance by the LRS procedure.

*Includes labelled and non-labelled Kepone.

containing waterhyacinth, a free floating hydrophyte with a massive root system, indicates that this species could remove substantial amounts of Kepone from contaminated waters, such as those of Bailey Creek, where Kepone concentrations are high due to alkaline pH (USEPA, 1978).

Accumulation of Kepone in Shoots and Roots

Large amounts of ^{14}C-labeling were associated with the roots and rhizomes of the four species (Figure II-2). The values for the controls and the three day incubations were significantly different by the LSR procedure. However, only smooth cordgrass showed any significant difference after the third day. Although the values for the three, five, and seven day incubations of American bulrush are not significantly different at the .05 level of significance, an increasing trend was observed at the .25 level of significance. The significance between the three day and seven day incubation of smooth cordgrass and the increasing trend noted with American bulrush indicate these plants may accumulate more Kepone in their roots over longer periods of exposure, as would be the case in the natural environment.

The dpm per gram dry weight which represents the maximum mass of Kepone which would be soluble in the water associated with a gram dry weight of plant tissue (calculated from plant dry weights and fresh weights) at pH 7 is indicated by an 'x' on the vertical axes of Figure II-2. Since

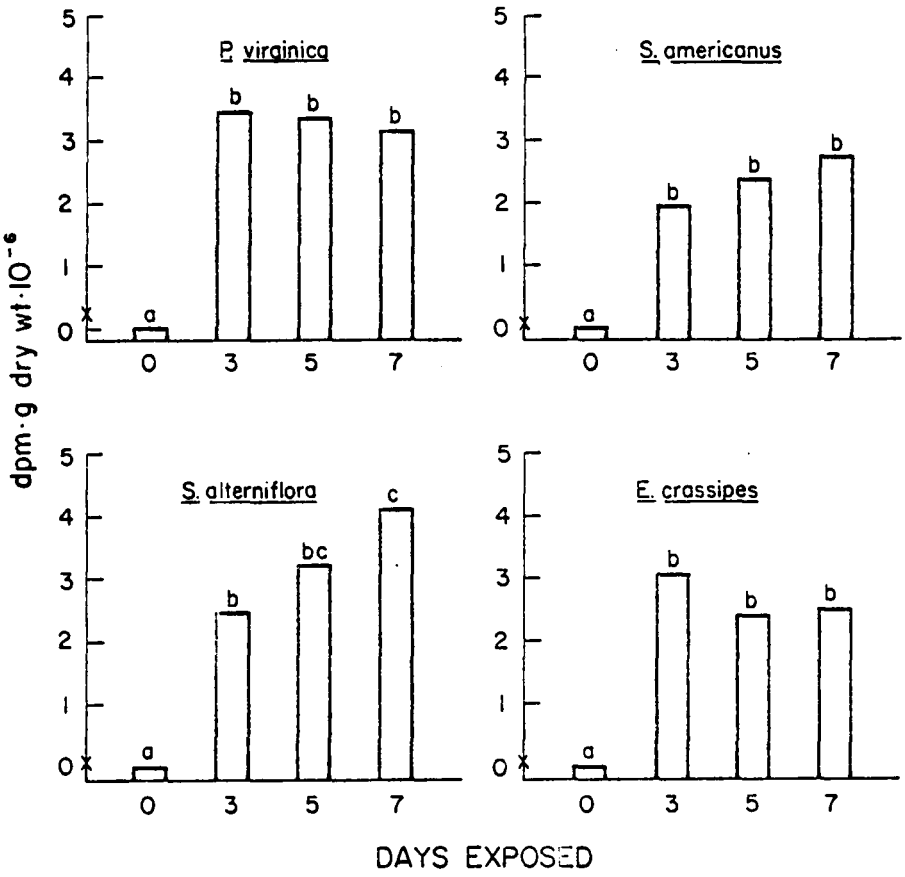


Figure II-2. Accumulation of radioactivity by roots of arrowarum (P. virginica), American bulrush (S. americanus), smooth cordgrass (S. alterniflora), and waterhyacinth (E. crassipes) exposed to ^{14}C-Kepone containing nutrient solution. Bars represent the average of three replications. Bars in individual graphs with the same letter are not significantly different at the .05 level of significance by the LSR procedure. The "x's" on the vertical axes indicate the dpm which represents the maximum mass of Kepone which is soluble in the water associated with the root tissue.

the amount of radioactivity associated with root tissue represents a mass of Kepone which far exceeds that which is soluble in the water associated with the root tissue, at physiological pH, it appears that the Kepone molecule is altered or bound. The magnitude of Kepone accumulation above that which could be in solution and the known affinity of Kepone for organic particulate matter (USEPA, 1978) leads to the conclusion that most of the Kepone associated with the plant roots was bound.

Translocation of the ^{14}C-label to the shoots was slight in all cases (Figure II-3). The amounts of radioactivity detected in the shoots of arrowarum, smooth cordgrass, and waterhyacinth were not significantly different from the controls until seven days of incubation. Due to the variability among bulrush plants within each treatment, significant differences could not be shown at the .05 level; however, a trend is evident at the .25 level.

Translocation of the ^{14}C-label does not, necessarily, indicate metabolic uptake of Kepone. However, since the steles of waterhyacinth roots are surrounded by an endodermis (Sculthorpe, 1967) and an endodermis is probably present in arrowarum, bulrush, and cordgrass roots, translocation does necessitate absorption into the symplast system before entry into the vascular tissue. That significant translocation was not observed until the longest incubation time indicates

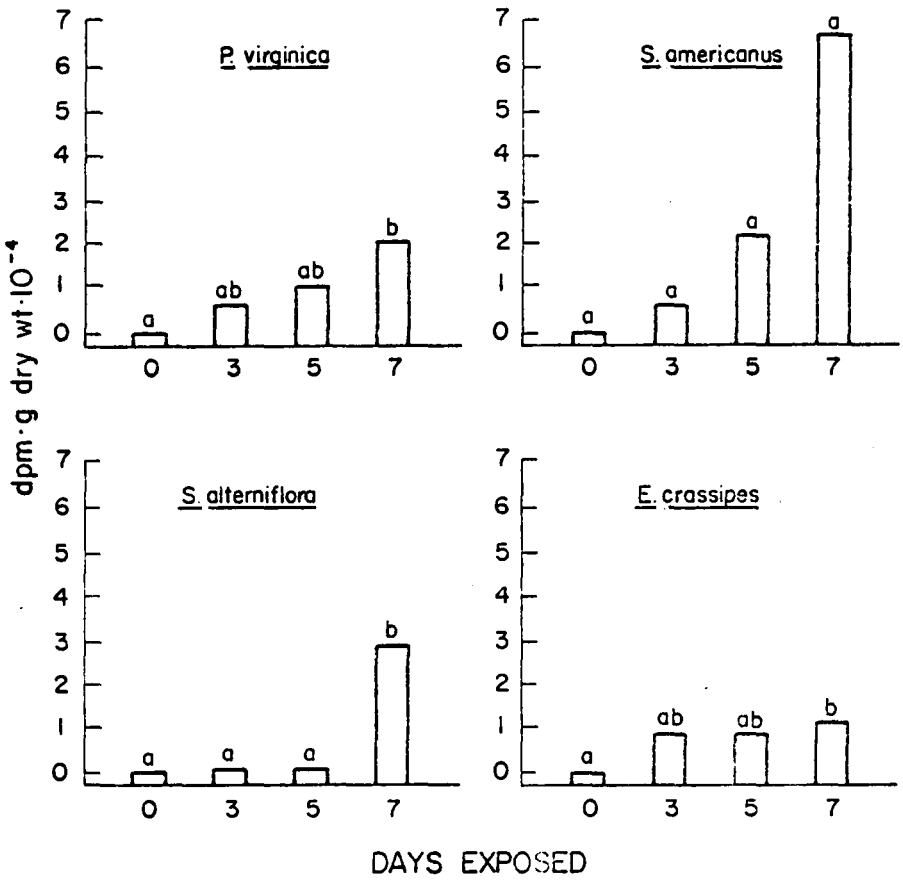


Figure II-3. Radioactivity accumulated in arrowarum (P. virginica), American bulrush (S. americanus), smooth cordgrass (S. alterniflora), and waterhyacinth (E. crassipes) shoots after exposure of roots to ^{14}C-Kepone containing nutrient solution. Bars in individual graphs with the same letter are not significantly different at the .05 level of significance by the LSR procedure.

that the root cell walls, Casparian strips, and plasmolemmas presented effective barriers to Kepone absorption. Since translocation was not observed until after plant roots ceased to accumulate radioactivity, it appears that absorption of Kepone was a slow process of passive diffusion which occurred, to a significant extent, only after adsorptive sites associated with root tissues were saturated. The possibility of root damage, which would allow for a direct pathway into the vascular tissue cannot be disregarded.

It must be realized, that from the data presented, the nature of the ^{14}C-labelled molecules associated with the plant tissues is not known. The presence of radioactivity in the plant shoots does not, necessarily, indicate that the parent Kepone molecule was absorbed and translocated. Via the action of non-specific enzymes associated with cell walls, the Kepone molecule may have been altered to a form which was more readily absorbed. This aspect will be addressed further in Section III.

After the longest incubation (seven days), the largest proportion of the initial radioactivity was associated with the roots (Figure II-4), with smaller proportions remaining in the treatment solution (roots had only slightly less with smooth cordgrass). Plant shoots contained only a small proportion of the initial label, with a maximum of 3%.

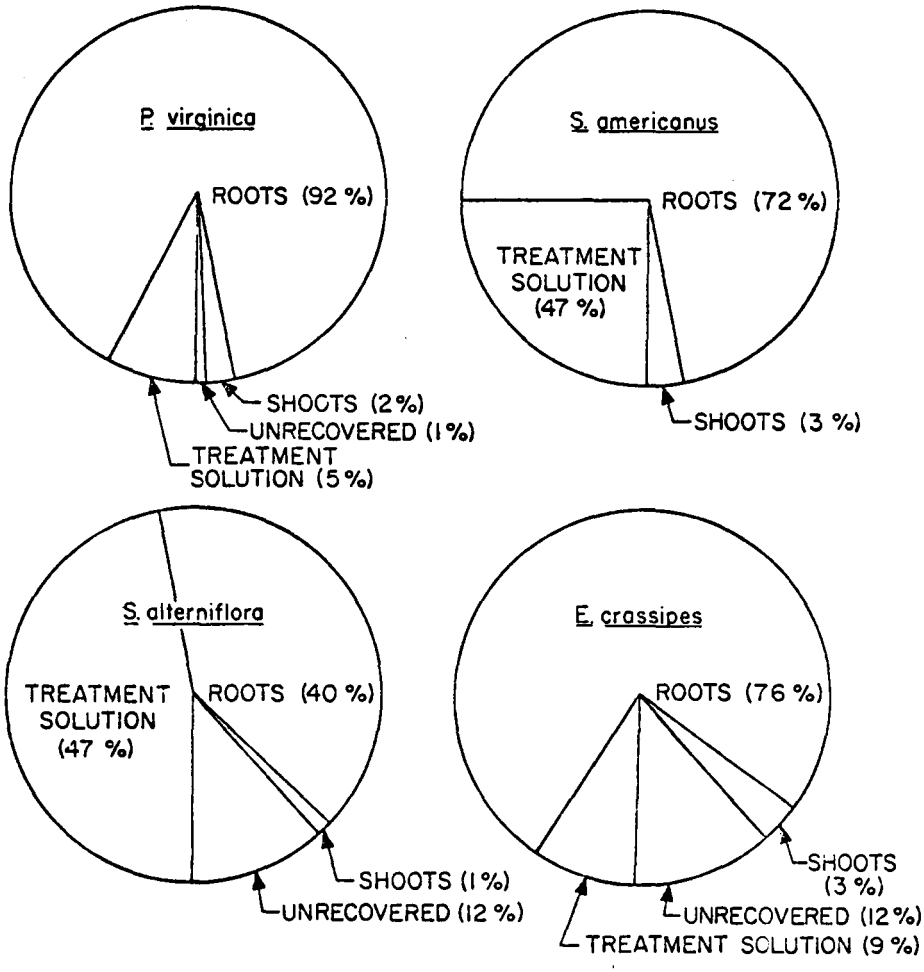


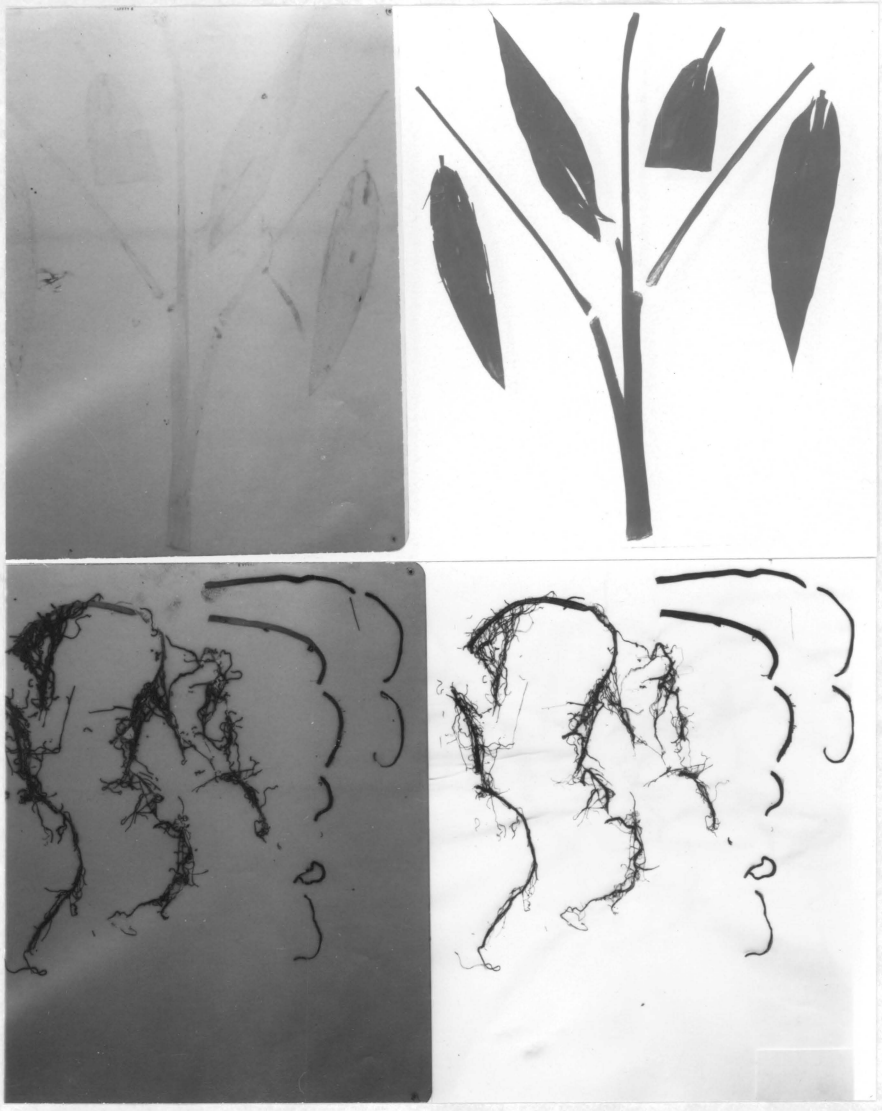
Figure II-4. Proportions of initial ^{14}C-label remaining in solution, and associated with the roots and shoots of arrowarum (P. virginica), American bulrush (S. americanus), smooth cordgrass (S. alterniflora), and water-hyacinth (E. crassipes) after seven days root exposure to ^{14}C-Kepone containing nutrient solution.

Recovery of radioactivity from arrowarum and bulrush was 99% or greater. Radioactivity which was not recovered from smooth cordgrass and waterhyacinth was probably lost when rinsing roots.

Distribution of Radioactivity in Plants

Autoradiographs of arrowarum, American bulrush, smooth cordgrass, and waterhyacinth are presented in Figures II-5, II-6, II-7, and II-8, respectively. The dark images obtained from roots, after only three days of autoradiography, and the slight images obtained from shoots after five weeks are in agreement with quantitative data obtained from combustion of plant material and LSC.

In general, radioactivity in both roots and shoots appears throughout the plant tissue, with no evidence of concentration in any individual sections. No movement of radioactivity from lower to upper shoot portions was detected between plants exposed for three, five, and seven days (autoradiographs of three and five day incubations not shown). Only in the shoot autoradiograph of American bulrush did it appear that higher radioactivity was present at the lower portion of the stem with diminishing radioactivity toward upper portions (Figure II-6). This would indicate temporal increases in shoots over longer exposure periods. It also appears in this autoradiograph that



A B

Figure II-5. Autoradiograph (A) of arrowarum plant (B) which was incubated for seven days in nutrient solution containing ^{14}C-Kepone.

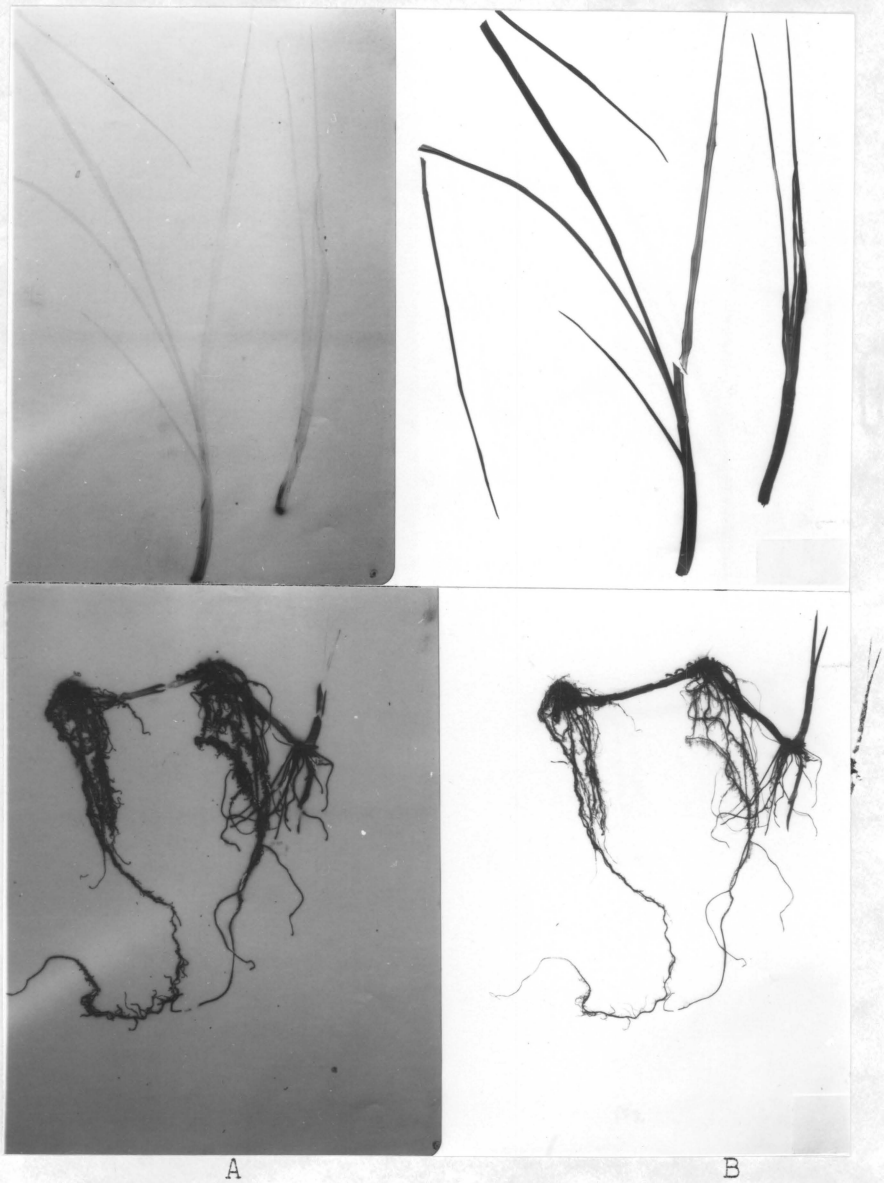
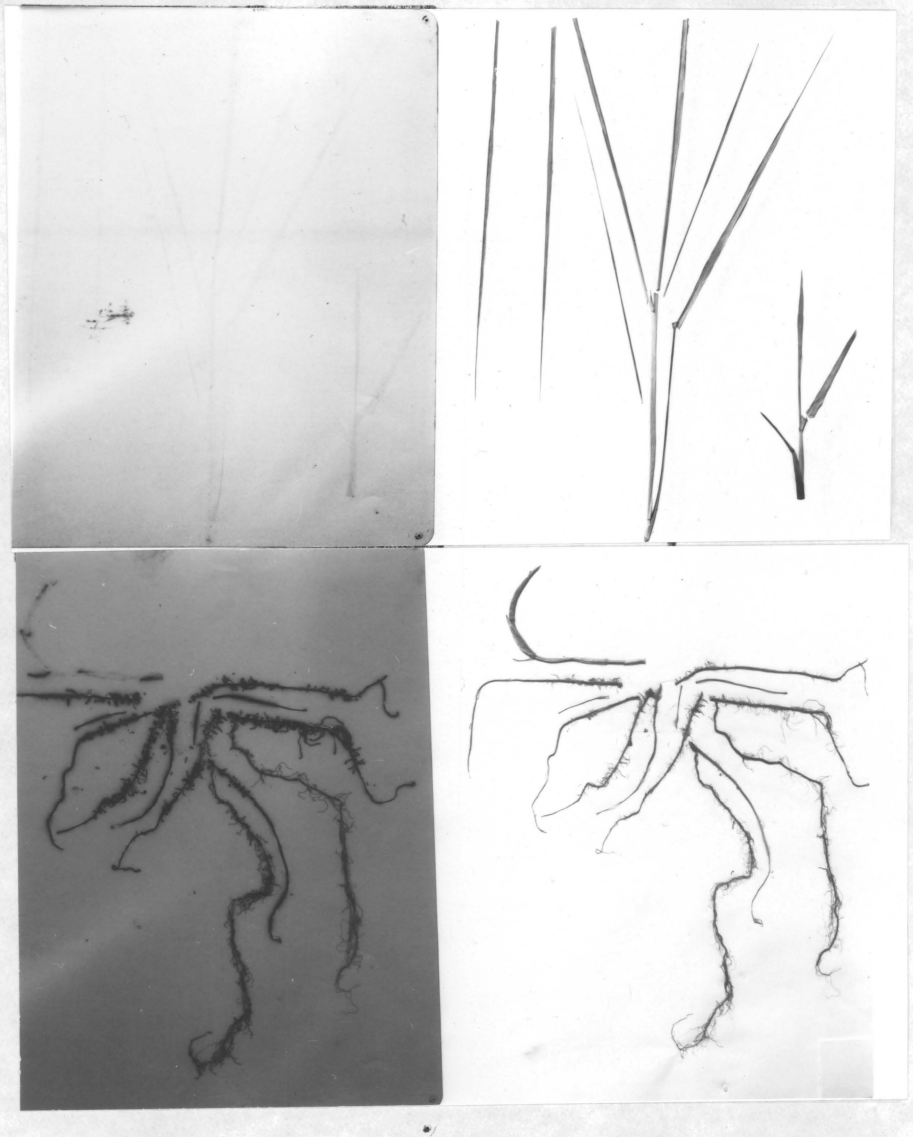
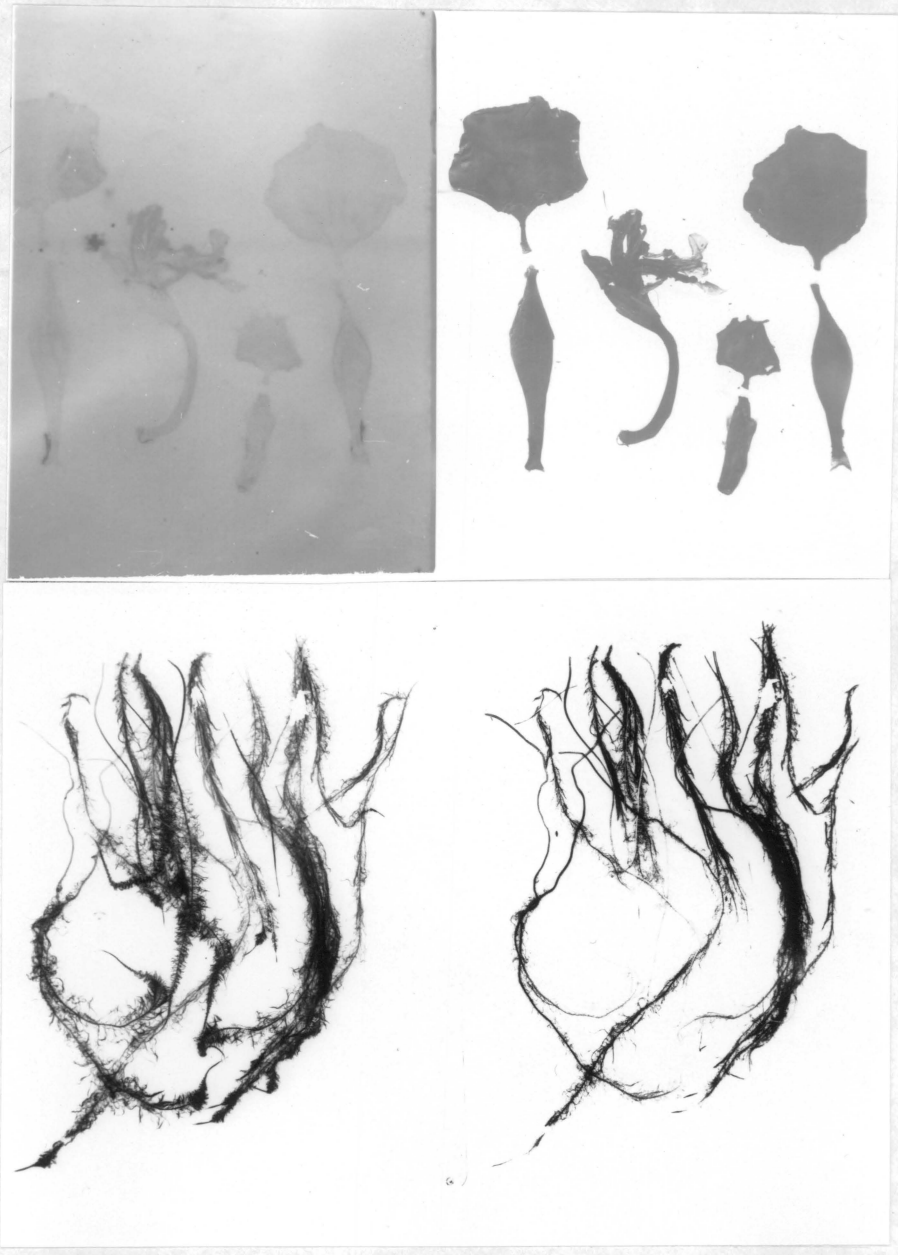


Figure II-6. Autoradiograph (A) of American bulrush plant (B) which was incubated for seven days in nutrient solution containing ^{14}C-Kepone.



A B

Figure II-7. Autoradiograph (A) of smooth cordgrass plant (B) which was incubated for seven days in nutrient solution containing ^{14}C-Kepone.



A B

Figure II-8. Autoradiograph (A) of waterhyacinth plant (B) which was incubated for seven days in nutrient solution containing ^{14}C-Kepone.

radioactivity was concentrated in the leaf sheath vasculature of the lower shoot portion.

The newly emerged shoot, which appears in the American bulrush root autoradiograph (Figure II-6) is enlarged in Figure II-9. A concentration of the ^{14}C-label is evident outlining the large epidermal cells of the leaf sheath which was submersed in the treatment solution during the seven day incubation. This indicates that Kepone is bound apoplastically. Since the Kepone molecule is polar, polar attractions of Kepone molecules with cell wall and middle lamella components can be expected. Although this concentration could not be distinguished in root images, the speculation may be made that similar binding is associated with root epidermal cells and cortical cells. This would indicate a resistance to absorption of Kepone by plant roots, and partially explain the very slight translocation which was observed after the plant roots ceased to accumulate Kepone.

Acknowledgement

Appreciation is expressed to Allied Chemical Company for ^{14}C-Kepone and technical Kepone which was supplied gratis.

Figure II-9. Enlargement from American bulrush autoradiograph (Figure II-6) showing concentration of ^{14}C-label outlining epidermal cells of leaf sheath.

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III. ANALYSIS OF FOUR HYDROPHYTE EXTRACTS FOR ^{14}C-KEPONE® AND METABOLITES

Abstract

Translocation of Kepone® (decachloro-octahydro-1.e 4-metheno-2H-cyclobuta [cd] pentalen-2-one) was demonstrated by identifying ^{14}C-Kepone in shoot extracts of arrowarum [Peltandra virginica (L.) Schott and Endl.], waterhyacinth [Eichhornia crassipes (Mart.) Solms] and American bulrush [Scirpus americanus Pers.], after root treatment. Additional ^{14}C-labelled spots were detected on chromatograms of shoot extracts from arrowarum, waterhyacinth, and American bulrush. ^{14}C-Kepone was also identified in root extracts of these plants and smooth cordgrass [Spartina alterniflora Loisel.]. Three additional ^{14}C-labelled spots were detected on root extract chromatograms of arrowarum, waterhyacinth, and American bulrush, and five in the roots of smooth cordgrass.

Introduction

Previous investigations have indicated that Kepone is resistant to microbial degradation (USEPA, 1978). Data submitted to EPA by Allied Chemical Company showed no significant decline of Kepone concentration in soil after 154 days (USEPA, 1978). Garnes (1977) reported that

Kepone was not degraded in model ecosystems using James River sediment. In similar studies at the Battelle Memorial Institute, degradation of Kepone was not observed in sediment from Bailey Bay, or in artificially contaminated cores from above Richmond (USEPA, 1978). Vind (1976) has shown that Kepone was not degraded in sea water when incubated both aerobically and anaerobically for 12 months. The only evidence of Kepone biodegradation was obtained with five mold cultures which grew with Kepone as the sole carbon source (USEPA, 1978). Metabolism or degradation of Kepone by higher plants has not been reported.

In the previous study, arrowarum [Peltandra virginica (L.) Schott and Endl.], American bulrush [Scirpus americanus Pers.], waterhyacinth [Eichhornia crassipes (Mart.) Solms], and smooth cordgrass [Spartina alterniflora Loisel.] were shown to accumulate ^{14}C when their roots were exposed to nutrient solution containing ^{14}C-Kepone. The present study was undertaken to determine if ^{14}C-Kepone is translocated by these hydrophytes, and to seek for evidence of metabolism of the chemical.

Materials and Methods

Plant Material

Stock solutions of ^{14}C-Kepone were prepared as described in Section II, Materials and Methods, and used to

prepare treatment solutions. Three plants each of arrowarum [Peltandra virginica (L.) Schott and Endl.], waterhyacinth [Eichhornia crassipes (Mart.) Solms], American bulrush [Scirpus americanus Pers.], and smooth cordgrass [Spartina alterniflora Loisel.] were prepared as previously described and incubated with their roots in the treatment solution for ten days. After incubation, the roots were rinsed thoroughly with tap water, roots and shoots separated (rhizomes included with roots), and stored at -15 C for no longer than three weeks before analysis. A 5.0 ml aliquot of the nutrient solution from each jar was stored at 15 C for no longer than one week before analysis.

Extraction

The extraction procedure used was similar to that of Hodgson et al. (1978) for extraction of Kepone from finfish. A 30 g fresh weight sample of chopped shoot tissue was ground to a coarse powder in a mortar and pestle with 90 g anhydrous Na_2SO_4. The powder was transferred to two 33 x 80 mm cellulose extraction thimbles and soxhlet extracted for 12 hours with 200 ml diethyl ether/petroleum ether (1:1 v/v). The same procedure was used for root tissue with 10 g fresh weight plus 30 g anhydrous Na_2SO_4, and extracted in one extraction thimble.

Nutrient solution samples were extracted by partitioning with diethyl ether/petroleum ether (1:1 v/v).

Purification of Extracts

Root and nutrient solution extracts did not require purification before chromatographic analysis.

Several methods were evaluated for purifying shoot extracts. These included modifications of the liquid/liquid partitioning used by Blanke et al. (1977), macro-Florisil®column chromatography, and modifications of the micro-Florisil column methods used by Hodgson et al. (1978). A modification of the latter proved most reliable for recovery of the ^{14}C-label and removal of co-extracted plant constituents.

Samples were prepared for purification by concentrating under vacuum, transferring a volume equivalent to 5.0 g tissue to a tapered centrifuge tube, and evaporating almost to dryness with air. The sample was dissolved in 0.5 ml diethyl ether/petroleum ether (1:1 v/v). A 14 x 300 mm glass column with a sintered glass bottom was packed with 6.0 g Florisil (60-100 mesh) plus 3.0 g anhydrous Na_2SO_4, and activated for 12 hours at 130 C. After cooling, the column was washed with 20 ml petroleum ether. The sample was placed on the column when the petroleum ether reached the top of the bed. The sample tube was rinsed with an additional 0.5 ml of the solvent, which was added to the column. Solvents I, II, and III were added consecutively to the column. Solvents I, II,

and III consisted of 20 ml petroleum ether, 40 ml methanol/acetonitrile/benzene/hexane (1:2:4:93 v/v/v/v), and 60 ml methanol/benzene (1:99 v/v), respectively. As the three solvents eluted from the column, the entire volume was collected. These will be referred to as Fractions I, II, and III. Radioactivity in each fraction was determined with a Beckman LS-250 liquid scintillation counter.

Chromatographic Analysis

Extracts were concentrated under vacuum, and spotted on 200 µm silica gel thin layer plates, which had been activated for three hours at 100 C. The plates were developed in one direction with methanol/chloroform (1:9 v/v) or acetic acid/diethyl ether/hexane (1:20:80 v/v/v), or in two directions with acetic acid/chloroform (1:9 v/v) and methanol/chloroform (1:9 v/v). Co-chromatographed standards consisted of extracts of untreated waterhyacinth plants to which ^{14}C-Kepone was added or, extracts of the ^{14}C-Kepone containing nutrient solution which had not been used to treat plants. ^{14}C-labelled spots were located on x-ray film, after exposure to the plates for two weeks.

Results and Discussion

Extraction

Since the major emphasis of this study was to verify the presence of Kepone in the plant shoots, analysis beyond

the ether extraction was not performed. A more detailed study of the metabolism of Kepone by higher plants should include a more complete plant tissue extraction.

Purification of Shoot Extracts

Since the amount of radioactivity in shoot extracts was low, as was expected from previous studies, a large volume of extract was concentrated for chromatographic analysis. The high concentration of plant constituents in the extracts interfered with the separation of components on the chromatographs and the plant extracts had to be purified.

Methods were not available for thin layer chromatographic analysis of Kepone in plant extracts. Therefore, methods for preparing other biological specimens for gas liquid chromatography (Blanke et al.,1977; Hodgson et al., 1978) were used with modification.

The liquid/liquid method used by Blanke et al. (1977) to purify stool and bile specimens was attempted with extracts from 5.0 g fresh weight of plant tissue. The method proved unsatisfactory for this amount of sample. By increasing the solvent volumes in the procedure, a relatively clean sample was obtained. However, with the increased volumes the procedure became rather cumbersome, and up to 30% of the initial radioactivity was lost in emulsions. For these reasons, and because the initial

acid hydrolysis produced less than optimum conditions for the detection of Kepone conjugates, the procedure was not used.

Different amounts of Florisil and varied solvent proportions in a 25 x 440 mm glass column proved inefficient in eluting the ^{14}C-label, and offered no advantage over the modification of the micro-Florisil column procedure of Hodgson et al. (1978).

The micro-Florisil column did not remove all interfering materials but yielded a satisfactory sample. The percent recovery from the column was always greater than 87% of the radioactivity in the crude extract, and usually greater than 90%. Solvent I eluted only negligible amounts of radioactivity (<0.1%). Solvents II and III eluted radioactivity, but the larger amount always occurred in Fraction III. Fraction III also contained the greatest amount of coextractants, the most obvious of which were chlorophylls.

Chromatographic Analysis

Extracts of treatment solutions which were exposed to plant roots and chromatographed with methanol/chloroform or acetic acid/diethyl ether/hexane did not reveal chromatographically different compounds.

Root extract chromatograms, developed with methanol/chloroform (Figure III-1), revealed one distinct ^{14}C-labelled

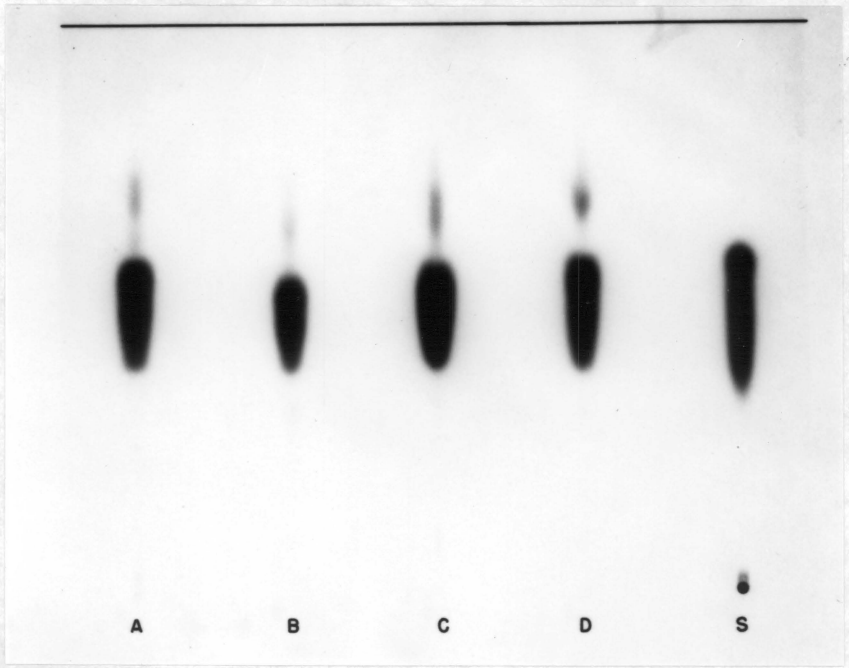

Figure III-1. Methanol/chloroform (1:9 v/v) developed chromatograms of root extracts from plants incubated for 10 days with ^{14}C-Kepone. A: arrowarum, B: waterhyacinth, C: American bulrush, D: smooth cordgrass, S: standard.

image other than that corresponding to Kepone. Other faint images were detected which were not present in the standard. However, these images were so indistinct that they were considered insignificant. Tailing also occurred at the major spot which may have masked further separation.

Better separation of root extracts was accomplished on chromatograms developed with acetic acid/diethyl ether/hexane (Figure III-2). Four chromatographically different images are evident in the arrowarum, waterhyacinth, and bulrush extracts with Rf values of .05, .09, .16, and .45. Six ^{14}C-labelled spots were evident on the smooth cordgrass chromatogram with Rf values of .05, .09, .16, .19, .45, and .60. Only the compound with Rf .05 (Kepone) was evident in the standard. A small amount of tailing did, however, occur with the standard between Rf .15 and Rf .20. It appears that a portion of the Kepone molecules associated with the roots was altered.

Development of chromatograms containing Fraction II and Fraction III with methanol/chloroform resulted in severe tailing of both the extracts and standard, and detection of individual compounds was impossible. The tailing was apparently a result of interference from chlorophylls since the location of radioactivity corresponded to the disperse green-colored tailing on the chromatogram.

Two dimensional development with acetic acid/chloroform

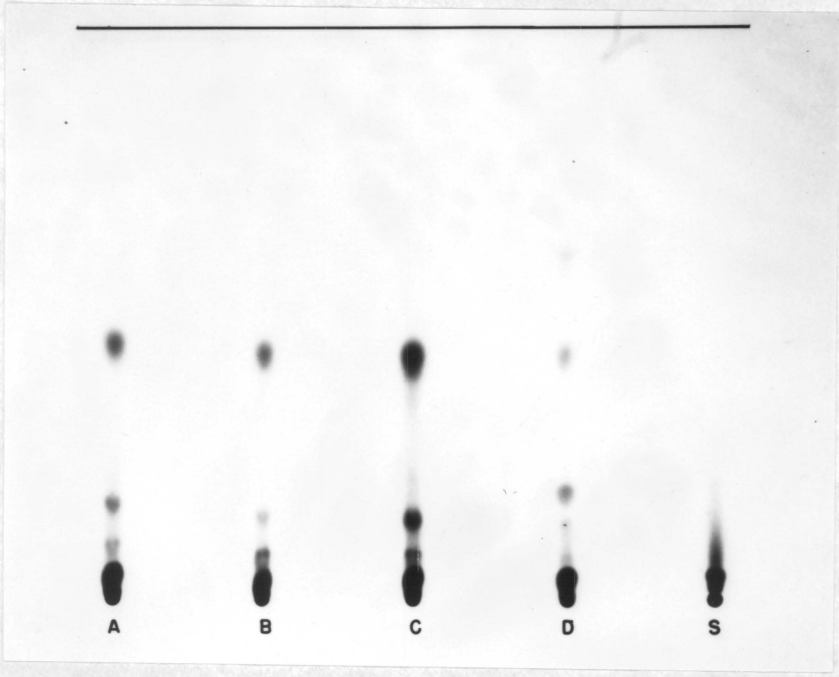

Figure III-2. Acetic acid/diethyl ether/hexane (1:20:80 v/v/v) developed chromatograms of root extracts from plants incubated for 10 days with ^{14}C-Kepone.
A: arrowarum, B: waterhyacinth, C: American bulrush, D: smooth cordgrass,
S: standard.

and methanol/chloroform resulted in better separation of Fraction III. The standard chromatograph developed in this manner (Figure III-3) resulted in a tailed spot corresponding to Kepone and a diffuse spot which was associated with chlorophylls. These spots were also detected on the Fraction III chromatograms of arrowarum (Figure III-4), waterhyacinth (Figure III-5), and bulrush (Figure III-6), which demonstrated that the parent Kepone molecule was translocated to some extent in these plants. Additional ^{14}C-labelled spots were detected on Fraction III chromatograms of treated plants, which were not evident on the standard chromatogram (Table III-1).

It was not within the scope of this study to determine the molecular identity of Kepone metabolites. Therefore, the identity of the ^{14}C-labelled spots, which were chromatographically different from Kepone, can only be speculated. Detection of these spots does not necessarily reflect an alteration of the Kepone molecule, but could be the result of conjugation or binding of the Kepone molecule with plant constituents. Molecular interactions of plant constituents with a polar molecule, such as Kepone, could be expected and would affect the movement of Kepone in the mobile phase. The most probable conjugation of the Kepone molecule would occur with the carbonyl oxygen. The Kepone molecule does form addition products with alcohols, amines (McBee et al., 1956) and ketones (Gilbert et al.,

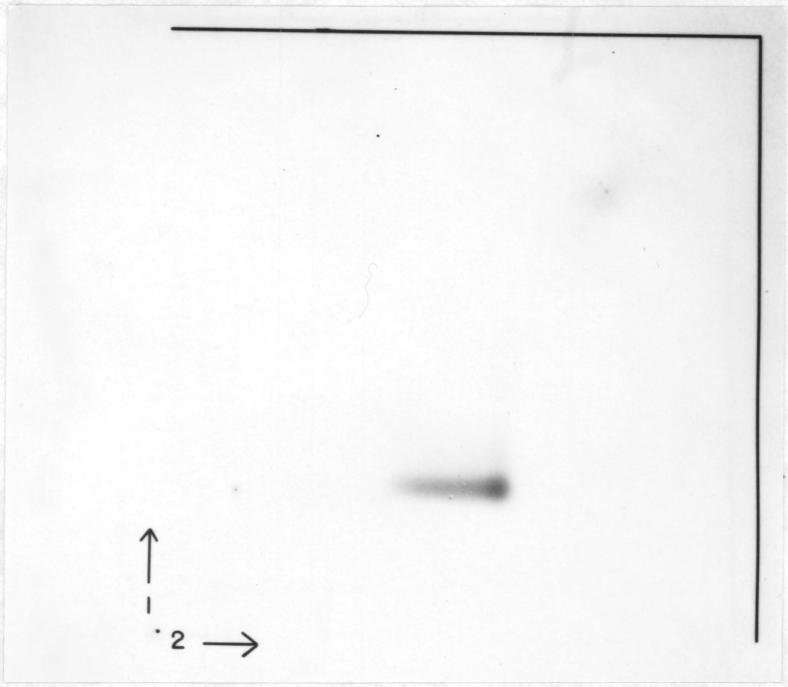

Figure III-3. Two dimensional shoot extract (Fraction III) chromatogram of standard.
(1) acetic acid/chloroform (1:9 v/v),
(2) methanol/chloroform (1:9 v/v).

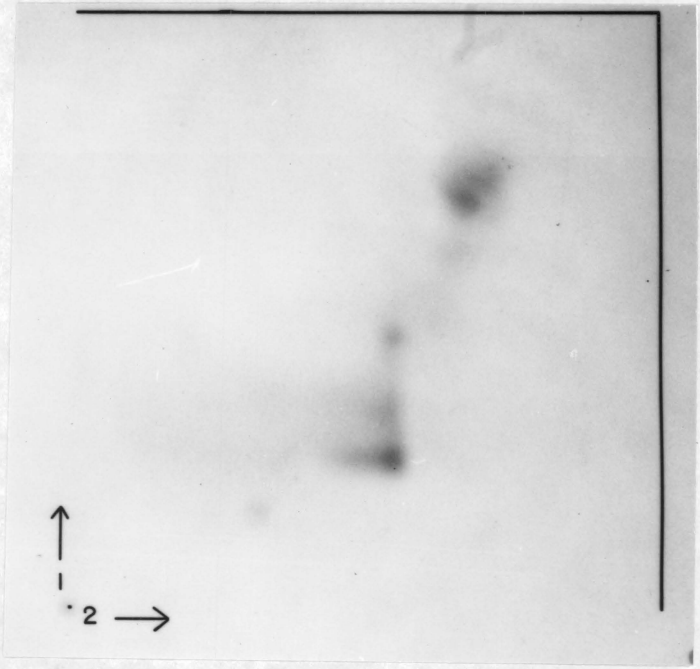

Figure III-4. Two dimensional shoot extract (Fraction III) chromatogram of arrowarum which was incubated for 10 days with ^{14}C-Kepone. (1) ascetic acid/chloroform (1:9 v/v), (2) methanol/chloroform (1:9 v/v).

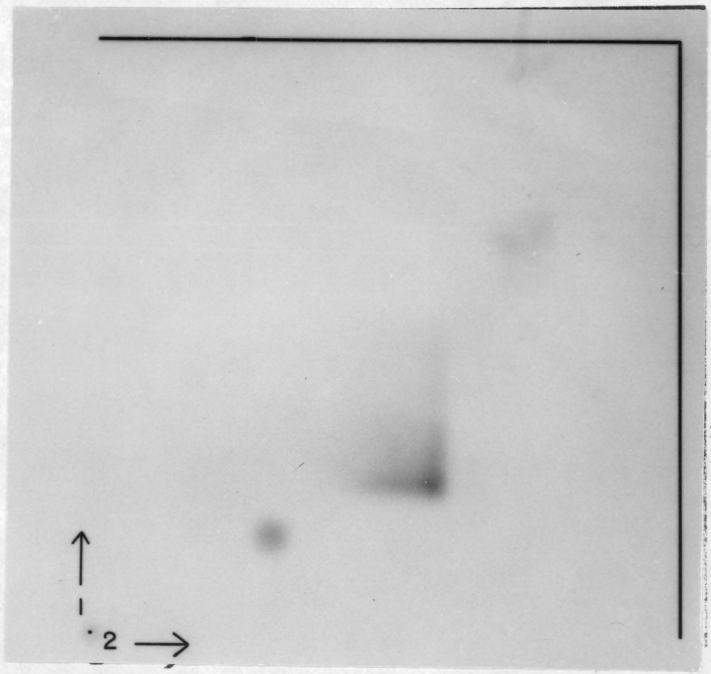

Figure III-5. Two dimensional shoot extract (Fraction III) chromatogram of waterhyacinth which was incubated for 10 days with ^{14}C-Kepone. (1) acetic acid/chloroform (1:9 v/v), (2) methanol/chloroform (1:9 v/v).

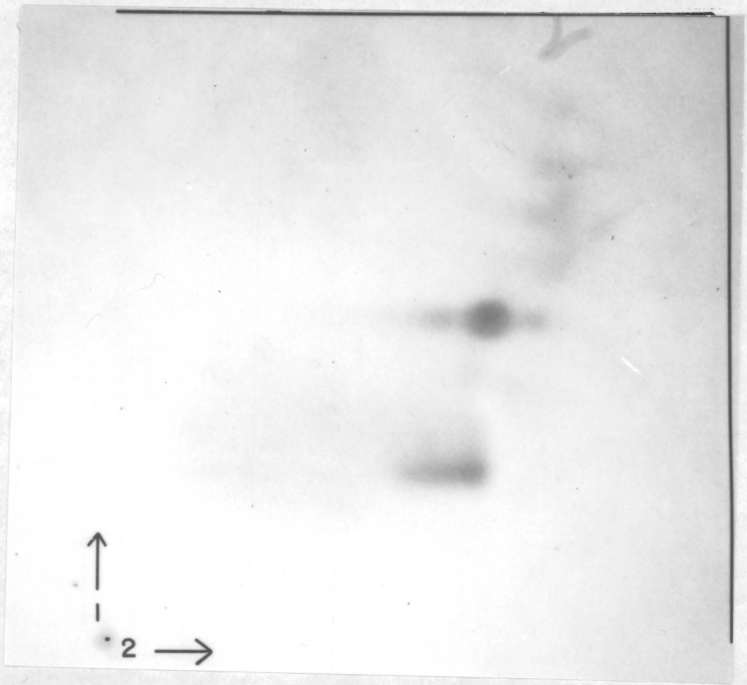

Figure III-6. Two dimensional shoot extract (Fraction III) chromatogram of American bulrush which was incubated for 10 days with ^{14}C-Kepone. (1) acetic acid/chloroform (1:9 v/v), (2) methanol/chloroform (1:9 v/v).

TABLE III-1. Rf values of ^{14}C-labelled spots detected on two dimensional chromatograms of Fraction III. "x" indicates presence of image on chromatogram.

Solvent, Rf		Chromatogram			
CH_3COOH / $CHCl_3$	CH_3OH / $CHCl_3$	arrow-arum	water-hyacinth	American bulrush	standard
.25	.54[1]	x	x	x	x
.66[2]	.66[2]	x	x	x	x
.17	.30	x	x		
.44	.57	x		x	
.44	.53			x	
.44	.70			x	

[1]tailed spot

[2]diffuse spot

1966a), and such additions could be expected with plant constituents. Since the addition products which have been studied have similar insecticidal activity (Griffin et al., 1964; Gilbert et al., 1966a; Gilbert et al., 1966b) and can be assumed to be at least as toxic to other organisms as Kepone, formation of such compounds in plants would be of little importance in Kepone mitigation, but would have environmental implications.

In demonstrating that Kepone is taken up by these plants under artificial conditions, a possible route for bio-translocation of Kepone in the environment is suggested. Further studies will be necessary to determine if environmental factors affect Kepone uptake by plants, and if other aquatic plants in the contaminated vicinity accumulate the chemical.

Acknowledgement

Appreciation is expressed to Allied Chemical Company for ^{14}C-Kepone and technical Kepone, which was supplied gratis.

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UPTAKE OF KEPONE® BY AQUATIC MACROPHYTES

by

Kenneth A. Langeland

(ABSTRACT)

Arrowarum [Peltandra virginica (L.) Schott and Endl.], waterhyacinth (Eichhornia crassipes (Mart.) Solms], American bulrush [Scirpus americanus (Pers.)], and smooth cordgrass [Spartina alterniflora (Loisel.)] removed Kepone® (dechachloro-octahydro-1,3,4-metheno-2H-cyclobuta[cd] pentelen-2-one) from water. ^{14}C-Kepone was translocated from roots and accumulated in small, but significant, quantities in the aerial portions of the plants. Approximately 80% of the ^{14}C-Kepone was removed from solution within three days by arrowarum, and waterhyacinth, however, very little additional Kepone was accumulated by these species after the initial three day exposure. Kepone uptake by the roots of American bulrush and smooth cordgrass appeared to continue for a seven day period. Translocation to the aerial portions of the plants was slow, with significant quantities reaching the shoot after seven days. Additional ^{14}C-labelled compounds were detected in shoot and root extracts, indicating possible metabolism of the Kepone molecule by these plants.