IAEA-TECDOC-306

RADIOTRACER STUDIES OF BOUND PESTICIDE RESIDUES IN SOIL, PLANTS AND FOOD

REPORT OF A RESEARCH CO-ORDINATION MEETING
ON ISOTOPIC TRACER-AIDED STUDIES
OF UNEXTRACTABLE OR BOUND PESTICIDE RESIDUES
IN SOIL, PLANTS AND FOOD
ORGANIZED BY THE
JOINT FAO/IAEA DIVISION
OF ISOTOPE AND RADIATION APPLICATIONS OF ATOMIC ENERGY
FOR FOOD AND AGRICULTURAL DEVELOPMENT
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FOREWORD

Current trends in population dictate intensified agricultural practices with concomitant growing use of agrochemicals, particularly in developing countries. Increased use of pesticides has greatly aided crop production, protected man from diseases, decreased losses of stored grains, and has generally improved man's welfare. Parallel to these trends, there is also a growing public sensitivity to contamination of the agricultural environment. Chemical residues inevitably find their way into human food and the environment.

Through the implementation of several programmes on isotopic tracer-aided studies on pesticide residues in food and the environment in the Joint FAO/IAEA Division, the complexity and apparent importance of the bound residue problem in environmental samples, food products and their intermediates have been illustrated.

The current programme was initiated in 1980 and was designed to help scientists of developing countries to make safe and effective use of isotope techniques for studying pesticide interactions with various components of the agricultural environment, under local conditions, particularly those pertinent to bound or "non-extractable" pesticide residues. The present report represents an appraisal of a group of studies with particular emphasis given to methodologies, bioavailability and possible adverse biological effects of bound pesticide residues.

Nuclear techniques have provided unique and powerful research tools in the study of these problems and the contributed research has clearly illustrated the potential value of these techniques.

Thanks to the generosity of the Swedish International Development Authority (SIDA), it has been made possible to undertake a number of these studies in developing countries.

CONTENTS

INFLUENCE OF AGRONOMIC AMENDMENTS ON THE FATE OF BOUND METHYL PARATHION RESIDUES IN SOILS

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Abstract

The fate of [ring-^{14}C]methyl parathion in a silt loam soil was monitored during a 49-day incubation period. At this point, 54% of the initial ^{14}C remained in the soil; of this, 13% was extractable with MeOH and 87% was bound residue. Soils were then treated with inorganic and organic amendments and incubated an additional 70 days. Release of methyl parathion bound residues could not be demonstrated, but mineralization of both bound and extractable ^{14}C to $^{14}CO_2$ was seen. Slow, continuous production of CO_2, all at comparable rates, occurred with the controls and with amendments H_2SO_4, $(NH_4)_2SO_4$, NH_4OH, chitin, oat seedlings, and oat straw. Glucose and asparagine caused high rates of $^{14}CO_2$ production. $HgCl_2$ gave very high initial rates of $^{14}CO_2$ loss; the rate declined to that of the control only after 9-10 weeks. The lime treatment exceeded the controls after 1 week, declining only slightly with time. The effects of sewage sludge and dairy manure were similar to the controls except that: (a) sludge caused a very high initial loss of $^{14}CO_2$, and (b) both treatments gave an unaccountable loss of ^{14}C, perhaps as $^{14}CH_4$ from anaerobic conditions. By 70 days, levels of extractable ^{14}C and bound ^{14}C had both declined twice as rapidly in certain amended soils as in unamended controls.

Introduction

For many years pesticides have been classified as either "persistent" or "nonpersistent" depending on the amount of the compound---with time---that could be extracted from soil, plant, or animal samples by conventional methods. Relatively recently, the use of ^{14}C-labeled chemicals has shown that, for many pesticides, a significant portion of the initially applied material may persist in an unextractable form designated as "bound residues", or BR.[1] Bound residues may refer to either the intact pesticide or compounds derived from it. In soils and plants, these pesticide BR may constitute a potential environmental problem (6). With the exception of a few thoroughly studied systems, little is known of the chemical nature of the BR, nor of their toxicity, bioavailability, accumulation potential, or ultimate fate.

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[1]At the First FAO/IAEA Research Coordination Meeting on Isotopic Tracer-Aided Studies of Bound Pesticide Residues in Soil, Plants and Food, in December 1981, the following definition of BR was adopted: "Nonextractable (bound) pesticide residues in soils, plants and food are chemical species originating from pesticide usage that cannot be extracted by methods commonly used in residue analyses and metabolism studies."

In typical agricultural practice, a pesticide is applied to a cropping system not in isolation, but often in concert with other treatments. Many of these other system components can affect the nature and rate of transformations undergone by the pesticide. Such components may be additional pesticides, organic and inorganic fertilizers, and soil amendments such as crop, animal, and environmental residues. Much of the past research has concentrated on the effects of such treatments on the formation of bound residues (1-3, 5-9,11). From an environmental standpoint, it is equally relevant to study any effects that these substances may have when applied to a soil already containing BR. In particular, we must be able to whether the BR will be released into the environment, becoming bioavailable and perhaps a hazard.

In order to evaluate the possibility of BR release caused by the addition of amendments, we undertook a laboratory study of the effect of selected agronomic practices (and related treatments) on the fate of ^{14}C-labeled bound methyl parathion (MP) residues in soils.

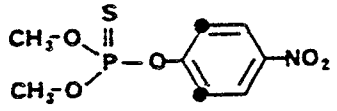
Materials and Methods

Soil. Duffield silt loam[1] (Ultic Hapludalfs) was freshly obtained from a grassy plot untreated with pesticides. The site was in Frederick County, Maryland. The Duffield series consists of well-drained soils developed from impure limestone.

The initial moisture content of the screened and sieved (2 mm) soil was 24.2%. Organic matter content is estimated at 5.2%, based on weight loss when heated to 380°C for 24 h. The pH = 5.9, measured as a 1:1 (w/v) slurry with distilled water.

Storage of the moist soil was in closed container at ambient temperature, in darkness.

Methyl Parathion. [ring-2,6-$C^{14}C$]Methyl parathion, specific activity 30 mCi/mMol or 114 µCi/mg, was obtained from Amersham/Searle. Thin-layer



chromatography on 250-µm silica gel plates eluted with $CHCl_3$:hexane:HOAc (8:2:1, v/v/v) gave a single spot at R_f = 0.91, which corresponded to that of unlabeled MP. This spot was scraped off and transfered to a scintillation vial; 1 ml of EtOAc and 10 ml Liquiscint cocktail were added. Based on liquid scintillation counting (LSC), 98.7% of applied ^{14}C was recovered, suggesting a purity of >99% MP.

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[1]Textural assignment is tentative, since particle size analysis is not yet done.

Pre-Amendment Incubation with Methyl Parathion. Duffield soil was treated with [^{14}C]MP and incubated in 44 chambers during the first stage of these experiments. Each chamber was a 16-oz glass jar, ca. 6.5 cm Ø X 14.0 cm high (internal dimensions) and 500 ml capacity. The wide-mouth jars had Teflon-lined screw caps. A liquid scintillation vial, 2.5 cm Ø, was cut to a height of 4.2 cm (volume ca. 21 ml) and affixed with silicone cement to the inside chamber wall, 7.5 cm above the bottom. The vial contained CO_2 trapping solution during incubation.

A stock solution of 75 mg MP (27.2 µCi) in 100 ml of acetone:H_2O (1:4, v/v) was used to treat the soil. To 186 g moist soil (150 g o.d. equiv.) in each chamber was added 2 ml of [^{14}C]MP solution. This was done in 25-µl increments by using a Hamilton Micro-Lab P microprocessor-controlled pipet; after every eight increments, the soil was thoroughly mixed. Then 10 ml of 0.1 N KOH was added to the trapping vial and the system was closed. Forty-four such incubation chambers were used; chamber #1 was an untreated control. All were incubated at 20°C in darkness. The moisture content was ca. 75% of the estimated field moisture capacity. The final concentration of MP was 10 ppm.

Immediately after treatment (i.e., Time 0), chambers #1-11 were sampled. Several small soil cores were taken from each until 25 g (o.d. equiv.) had been removed. The remaining soil was again mixed and set aside for incubation. Of the 25-g sample, ~19 g was Soxhlet extracted with 125 ml MeOH for 24 h; a 1-ml aliquot of the extract was analyzed by LSC to determine extractable ^{14}C. Air-dried subsamples (0.4-0.8 g) taken before and after extraction were combusted in a Packard Model 306 oxidizer, then counted to determine initial total ^{14}C an residual ^{14}C (i.e., bound residues). Finally, 3- to 4-g subsamples were used to determine moisture content at the time the chambers were sampled.

The remaining chambers were sampled in the same way according to the following schedule: #12-22 (14 days), #23-33 (29 days), and #34-44 (49 days). At this point, the pre-amendment experiment was completed.

Throughout the first 49 days, evolved $^{14}CO_2$ was measured in all chambers at 3-4 day intervals. Duplicate 1-ml aliquots were counted in 10 ml Liquiscint cocktail. The remaining KOH solution was discarded and replaced.

Incubation with Soil Amendments. At the 49th day after [^{14}C]MP treatment, the remaining soil was treated with various amendments, then further incubated. These treatments are listed in Table 1. The moisture content of chamber #1 had decreased, in 49 days, from 25.4% to 24.4%. On this basis, all treatments except "Control" had an additional 3.5 ml H_2O (or chemical solution) added. After amending the soils, the average moisture content was 26.8 ± 0.7%, slightly more moist than at Day 0. Three chambers were used per treatment.

Lime represented addition of powdered agricultural limestone (53.5% CaO + 1.5% MgO). For the acid amendment, 3.5 ml of 2.5 N H_2SO_4 was added to the 125 g soil remaining in each chamber. Chitin (poly-N-acetylglucosamine) was commercially available; its molecular weight was 771 and its composition of C, H, and N was 46.8, 6.5, and 7.3%, respectively. Oat seedlings grown for 3 weeks had been allowed to desiccate in situ; the tissue was further dried at 60°C and

Table 1. Amendments added to methyl parathion-treated soil after 49-day incubation

Treatment	Rate, per 100 g soil		
Control	---		---
Water (Moist control)	---		2.8 ml
Lime	2000 mg	+	"
Acid (H_2SO_4)	7.0 meq	+	"
NH_4OH	1220 mg	+	"
$(NH_4)_2SO_4$	48 mg	+	"
$HgCl_2$	28 mg	+	"
Asparagine	322 mg	+	"
Chitin	322 mg	+	"
Glucose	322 mg	+	"
Oat seedlings	322 mg	+	"
Straw	322 mg	+	"
Dairy manure	2560 mg	+	"
Sewage sludge	2560 mg	+	"

ground to a fine powder in a Wiley mill. Straw was finely ground mature oats. Dairy manure (containing no straw or bedding material) was lyophilized, ground to a fine powder (0.85 mm), and stored at -15°C until used. Sewage sludge (60% primary, 40% secondary) from Blue Plains Waste Treatment Plant at Washington, DC was treated in the same manner. All amendments were soil-incorporated as dry materials except for the 3.5-ml solutions of H_2SO_4, NH_4OH, and $HgCl_2$.

All flasks were sampled for $^{14}CO_2$, as before, at 3-4 day intervals. Soil samples were removed at 7, 21, 42, and 70 days following amendment. These were analyzed for total, Soxhlet-extractable, and residual (bound) ^{14}C by the procedures used before. At 70 days, soil pH was measured.

Gas Chromatographic Analysis. At 70 days post-amendment, most treatments were batch extracted: 7.5 g (moist soil) + 15 ml of acetone:benzene:MeOH (1:1:1, v/v/v) was shaken for 0.5 h, then centrifuged; the extraction was repeated. The volume of the combined extracts was reduced in a rotary evaporator, at 40°C, and the remaining organic solvents removed in a N_2 stream. Then 5 ml of hexane was added, with shaking, and the volumes of aqueous and organic phase recorded. LSC analysis was done with 0.5 ml of each phase. The hexane solutions were analyzed by GC, using a Hewlett-Packard Model 5880A gas chromatograph with a NP detector. The 1.8-m X 2-mm (i.d.) glass column contained 3% SP2100 on 80/100 Chromosorb WHP. Run temperatures were: injector (250°), column (190°), and detector (250°C). The carrier was helium, at 29 ml/min. The retention time of MP was 2.80 ± 0.04 min. Quantitation was by integrated peak area.

Results

Pre-Amendment Incubation with Methyl Parathion. The treated soils were analyzed for ^{14}C content in the whole soil, MeOH extracts, extracted soil, and evolved CO_2, at intervals to 49 days. A summary of these results is found in Table 2. Further comment appears in the Discussion section.

Table 2. Distribution of ^{14}C during a 49-day incubation of [^{14}C]methyl parathion.

Time, days	Radioactivity, dpm/g (%)			
	$^{14}CO_2$	Extractable	Bound	Total
0	0	7594	74	7668
	(0)	(99)	(1)	(100)
14	1914	606	3665	6185
	(25)	(8)	(48)	(81)
29	2115	576	3626	6317
	(28)	(8)	(47)	(82)
49	2350	527	3562	6439
	(31)	(7)	(46)	(84)

Incubation with Soil Amendments. Figure 1 depicts the changes observed in the evolved $^{14}CO_2$, extractable, and bound phases for several treatments. In general, the amount of evolved $^{14}CO_2$ increased with time, indicating that mineralization of methyl parathion and/or its metabolites was occurring. For $HgCl_2$-, glucose-, and asparagine-amended soils, the $^{14}CO_2$ evolved was nearly twice the ^{14}C present initially in the extractable phase.

The extractable fraction decreased in all treatments, but the decrease was always less than the corresponding $^{14}CO_2$ evolved so that some of the ^{14}C activity in these two fractions must come from the initially bound fraction. This fraction is seen to decrease with time as expected. The data in Table 3 for all the treatments show that with the exception of manure- and sludge-amended soils, the total ^{14}C recovery after 70 days was 3908 ± 153 dpm/g, which is nearly identical to the initial ^{14}C present in the soils, 4090 ± 146.

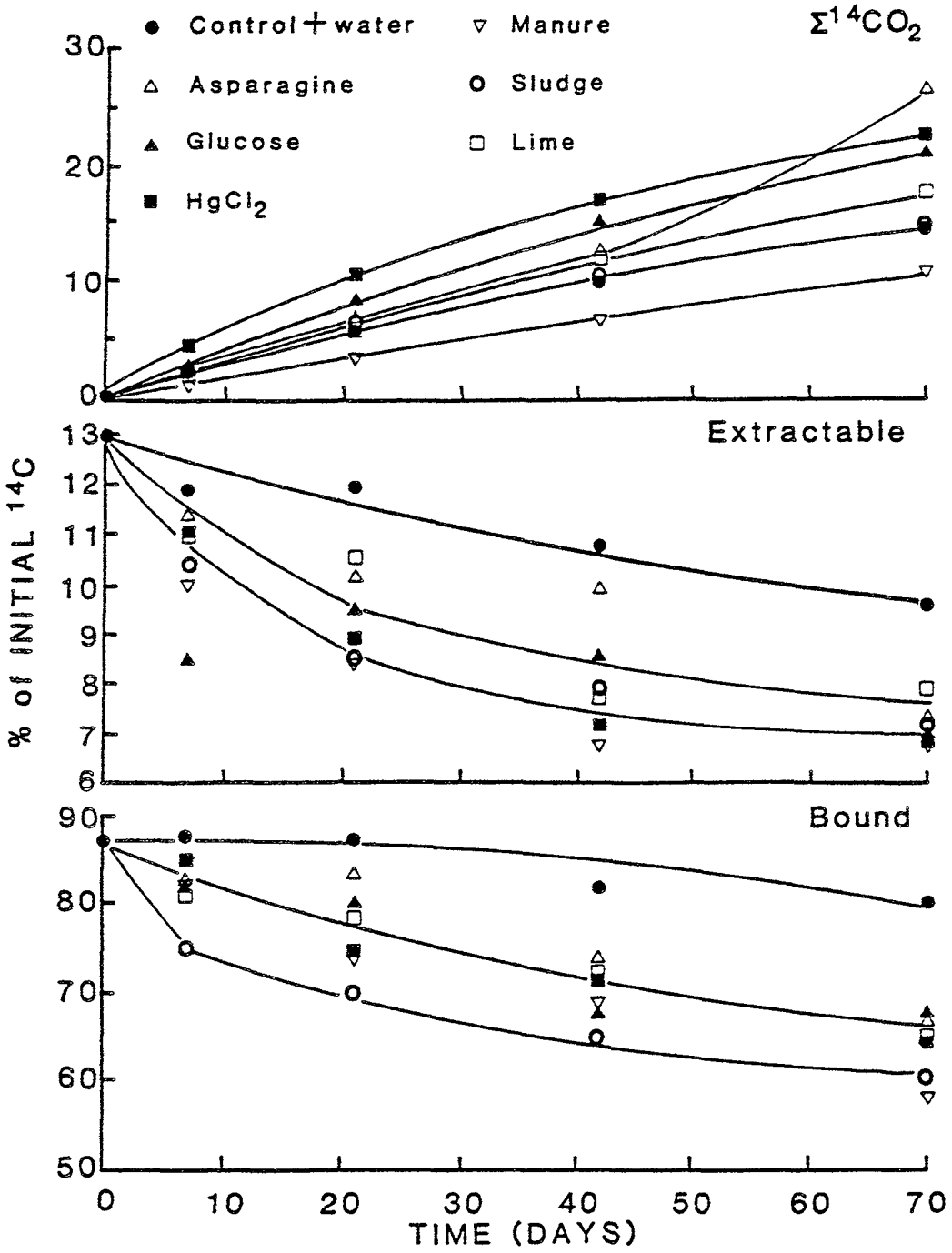

Figure 1. Distribution of ^{14}C from [^{14}C]methyl parathion following soil amendment on the 49th day after pesticide application.

Table 3. Distribution of radioactivity 119 days after soil application of [^{14}C]methyl parathion and 70 days after addition of soil amendments.

Treatment	Radioactivity, dpm/g ± SD			
	Cumulative CO_2	Extractable	Bound	Total
Control	429 ± 20	389 ± 17	2907 ± 87	3726 ± 24
Control + water	582 ± 8	391 ± 4	3280 ± 121	4253 ± 88
Lime	717 ± 17	332 ± 33	2683 ± 124	3764 ± 52
Acid (H_2SO_4)	551 ± 7	339 ± 23	2878 ± 169	3767 ± 64
NH_4OH	505 ± 16	376 ± 11	2951 ± 220	3825 ± 134
$(NH_4)_2SO_4$	467 ± 5	395 ± 4	2949 ± 160	3811 ± 47
$HgCl_2$	915 ± 36	272 ± 6	2634 ± 128	3819 ± 68
Asparagine	1070 ± 21	303 ± 23	2726 ± 166	3994 ± 23
Chitin	535 ± 3	375 ± 11	3103 ± 63	4013 ± 50
Glucose	842 ± 83	286 ± 22	2788 ± 167	3938 ± 100
Oat seedlings	586 ± 5	343 ± 6	3069 ± 115	4029 ± 131
Straw	616 ± 36	358 ± 22	2967 ± 52	3942 ± 49
Dairy manure	440 ± 7	280 ± 7	2375 ± 232	3102 ± 76
Sewage sludge	595 ± 23	294 ± 16	2442 ± 78	3334 ± 39

Discussion

The system chosen for study, methyl parathion, was chosen because it is a system in which the binding mechanism and degradation pathways are well known (3, 5, 7). In MP, the $-NO_2$ moiety is microbially reduced to $-NH_2$, yielding amino-MP, which is strongly bound to soil colloids. Degradation also proceeds via hydrolysis of the phosphate ester bond, which gives p-nitrophenol (or aminophenol) and diethylthiophosphate. The phenolic metabolite is further degraded to CO_2 and simpler organic molecules. Any $^{14}CO_2$ trapped in our experiments is the result of the degradation of MP and its phenolic metabolite.

The initial $^{14}CO_2$ activity in the soil was 7668 ± 590 dpm/g as determined by Soxhlet extraction immediately after preparation. As seen in Table 2, even after only a very short contact period 74 dpm/g (just under 1%) was already bound to the soil colloids and unextractable by Soxhlet extraction with MeOH. By 14 days the system contains primarily bound MP residues (50% of the added MP) with a much smaller extractable phase. The mass balance with the $^{14}CO_2$ is not complete and is most probably due to low trapping efficiency of $^{14}CO_2$ during the first 3.5 days of incubation during which the large amounts of CO_2 produced neutralized the 0.1N KOH. Between 14 and 49 days, the mass balance of ^{14}C activity is fairly constant, which indicates that trapping efficiency was no longer a problem. After 49 days of incubation, amendments were applied to a soil which contained 87% of its total activity in the form of bound residues.

The effect that different treatments had on the BR is quite striking. The addition of water alone was enough to significantly increase the amount of $^{14}CO_2$ mineralized (Table 3). The rate of $^{14}CO_2$ evolution in the watered control was consistently greater than in the unwatered control, which exhibited the same general pattern, i.e., a slow decline in the rate of mineralization with time, possibly due to the gradual decrease in soil moisture content (Fig. 2). The moisture contents of the watered and unwatered samples when the soil amendments were added were 26.9 and 23.4%, respectively, and decreased to 23.8 and 21.3% after 70 days of incubation. Ou et al. (10) found that methyl parathion degradation was also affected by soil moisture content, but only as the soil dried to about 30% of its moisture content at field capacity. In our study, the soil did not dry to such low values (FC = 34%, w/w). However, we are not dealing with degradation of freshly applied MP as were Ou et al., but rather with BR and so the importance of moisture content in such a case may be more critical.

Addition of certain amendments to MP-BR soil had little or no effect on the fate of the BR in the soil. The rate of $^{14}CO_2$ evolution from the soil amended with mature oat straw was identical to that of the watered control with the exception of the first sampling (1 day), in which the rate in the straw-amended soil was twice that of the control. This initially high rate may be the result of readily decomposable material in the straw which, when fully decomposed, leaves little substrate for increased microbial activity. Young oat seedlings, on the other hand, had a more pronounced initial effect lasting over 2 weeks, after which the rate actually decreased slightly below that of the watered control. The final (70 days) distribution of ^{14}C in these soils was not, however, different as seen from the results of Table 3. Addition of chitin, acid or N-containing fertilizers also had no effect on the metabolism of the BR. Lichtenstein et al. (8) found that $(NH_4)_2SO_4$ reduced the amount of parathion which was hydrolyzed to p-nitrophenol, but had little or no effect on the amount which was bound to the soil when planted to oats in an open system for 14 days. In a closed system $(NH_4)_2SO_4$ increased $^{14}CO_2$ production and binding of parathion, in contrast to our present results, indicating that the effects of amendments differ if applied together with a pesticide or to a soil containing BR.

In regions where soil pH values are low, it is a common agronomic practice to lime the soil to raise the pH to a value which will not inhibit plant growth. Addition of lime to the soil containing BR raised the pH from 5.9 to 7.6 after one week, where it remained for the duration of the experiment. The effect of the liming was pronounced mainly on $^{14}CO_2$ production, increasing it by nearly 25%. The $^{14}CO_2$ evolution exhibited two peaks, one at 8 days and the second at 22 days. Both these peaks correspond to days immediately following sampling and mixing of the soils in the flask. It would seem, therefore, that liming produced some volatile compound (NH_3) which had a slight inhibitory effect on the microbial activity and which was removed from the flasks upon sampling and mixing.

$HgCl_2$ was added as a soil sterilant although it can reach the soil from polluted industrial waste water as well. At the rate applied (280 µg/g) we would expect considerable inhibition of microbial processes in the soil, yet we

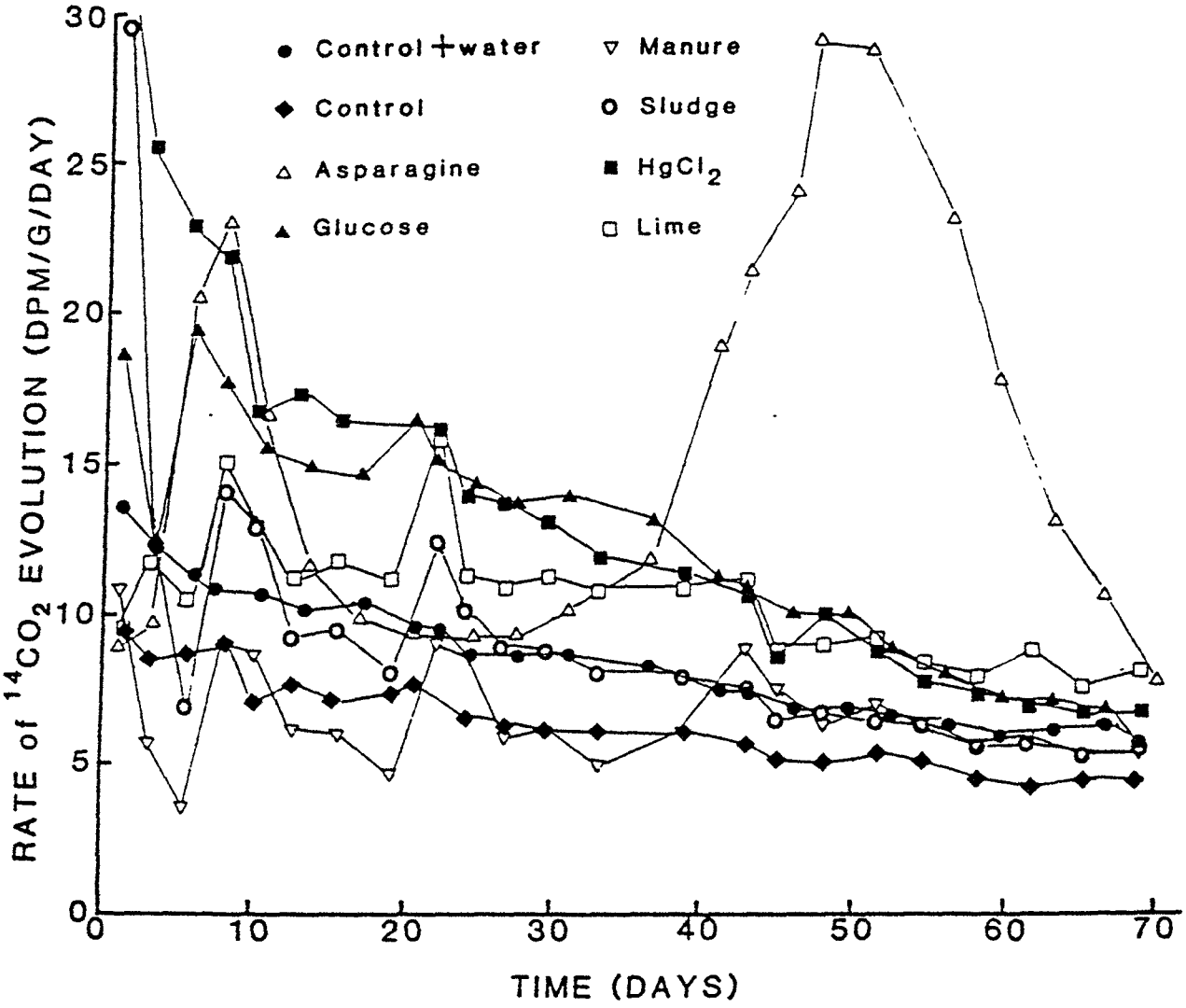

Figure 2. Daily production of $^{14}CO_2$ from [^{14}C]methyl parathion-treated soil as a function of soil amendments.

find that the rate of $^{14}CO_2$ evolution is initially three times greater than that of the watered control and continues to exceed it even after 70 days, so that mineralization of the BR to $^{14}CO_2$ is 60% greater in the $HgCl_2$-amended soil than in the control soil. One possible explanation for this is that the $HgCl_2$ did not affect the entire microbial population, leaving a small number of microorganisms which can utilize the biomass of the destroyed microbes as an energy source. Similar effects have been noted by Jenkinson and Powlson (4) for soils fumigated with $CHCl_3$, and has been used as the basis of their method for measuring soil biomass.

Two of the amendments added, glucose and asparagine, were chosen as models of readily available energy sources for the soil microbes, the former supplying only C and the latter supplying both C + N. Both the glucose- and asparagine-amended soils produced significantly greater quantities of $^{14}CO_2$ than did the watered control and also had lower extractable and bound phases after 10 weeks, indicating greater overall mineralization. The two treatments differ, however, in the way the rate of $^{14}CO_2$ evolution changes with time. For glucose, after an initial decrease, the rate increases again to nearly twice

that of the watered control and then slowly decreases over 70 days. The asparagine-treated soil has an initial rate lower than that of the watered control and exhibits two peaks in the rate of $^{14}CO_2$ evolution at 8 and 50 days. It is not quite clear at this time why these soils behave as they do and this point is presently being investigated further.

Sludge and manure are added to soils as amendments and as a means of disposal. Doyle et al. (1) found that these amendments altered the rate of pesticide degradation; Lichtenstein et al. (8) observed that these treatments produced less $^{14}CO_2$ and more bound residues when applied to soils together with parathion. Our results indicate that sludge has no effect on $^{14}CO_2$ production, but that manure reduces it by 25%. In both cases, a mass balance of the ^{14}C activity shows a loss of about 22% of the initial ^{14}C present at the time of treatment. Examination of the change in the rate of $^{14}CO_2$ evolution shows that both soils exhibited rapid and significant decreases in $^{14}CO_2$ evolution during the first week. After sampling and mixing, the rate increased only to decrease again until the next sampling and mixing. It is concluded, therefore, that the large amounts of decomposible organic material applied to the closed system resulted in O_2 depletion and the formation of anaerobic conditions with the possible loss of $^{14}CH_4$. Sampling and mixing replenished the O_2 supply which resulted in the increased rate of $^{14}CO_2$ production.

Summary

The results indicate that bound residues of methyl parathion are not easily released but are slowly mineralized to CO_2, and that many of the treatments studied could affect the rate at which MP was mineralized.

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STUDIES ON BOUND RESIDUES OF ^{14}C-MALATHION IN SOIL

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Abstract

The extractability and formation of bound ^{14}C-labelled residues in clay loam soil under laboratory conditions were investigated with malathion. ^{14}C-malathion rapidly decomposed to $^{14}CO_2$. Twelve days after treatment 56% of the applied dose was lost as $^{14}CO_2$. Methanol gave the highest extraction efficiency; 6% of the applied radiocarbon was extractable while bound residues amounted to 38%. The soil containing ^{14}C-labelled residues was fractionated into humic acid, fulvic acid and humin fractions. These fractions contained 7.83%, 16.81% and 19.36%, respectively of applied radiocarbon.

INTRODUCTION

Bound or non-extractable pesticide residues have become a concern of residue chemists only in the last 15 years. Bound pesticide residues are, basically residues remaining in soils and plants after exhaustive solvent extraction and have been detected for all classes of chemicals investigated so far; however, their percentage levels differ with variety of pesticide.

Several investigators have demonstrated the significance, release and plant uptake of soil bound residues(1-11). A recent study(12) has demonstrated that bound pesticide residues are susceptible to biodegradation by soil microbes. The availability of radiolabelled pesticides and the use of nuclear and combustion

or strong hydrolytic and pyrolytic techniques has made it possible to release, detect and quantify the radiolabelled bound residues without performing other analytical techniques.

Organophosphates (parathion, methyl-parathion, fonofos, fenitrothion, phorate, phosalone, RH-0994 and EPN) form considerable amounts (18-80%) of bound residues(13-20). Malathion, a widely used organophosphorus pesticide, was chosen for this investigation. The objective of the study reported herein was to examine, under laboratory conditions, the formation of bound residues of ^{14}C-malathion in soil and to determine the distribution of the residual ^{14}C among the soil organic matter fractions.

MATERIALS AND METHODS

Chemicals: Malathion (S-1,2-di (ethoxycarbonyl) ethyl 0-0-dimethyl phosphorodithioate) labelled at the 2,3 positions of diethyl maleate (specific activity 45.5 µCi/mg) was purchased from the Amersham International, England. Radiopurity was 98% as checked by TLC. Analytical grade malathion was obtained from American Cyanamid Co., Princeton, NJ. All other chemicals used were of analytical grade and redistilled solvents were used throughout this investigation.

Experimental Setup: Duplicate moist soil samples (100 g on oven-dry weight basis), having the following physicochemical characteristics: (source, NIAB field; pH (saturation paste), 7.9; saturation %age, 32.8; organic carbon, 1.8%; nitrogen, 0.22%; clay, .18%; silt, 28%; sand, 54%; texture, clay loam) were placed in Erlenmeyer flasks (250 ml) to which 2 ml of benzene containing 17.74 µg of ^{14}C-malathion (0.81 µCi) and

982.26 µg of unlabelled malathion were added to give insecticide concentration of 10 mg/kg. The solvent was evaporated and the soil was thoroughly mixed. The flasks were closed with stoppers having glass-cups containing 3 ml of 10% NaOH solution for trapping evolved $^{14}CO_2$ and incubated at 30°C for 12 days. Distilled water was added to the soil on alternate days to maintain the moisture content at 60% water holding capacity.

Determination of $^{14}CO_2$ Losses: The alkali was replaced at intervals of 2,4,8 and 12 days with fresh NaOH. Aliquots of the samples were analysed for ^{14}C by liquid scintillation counting.

Determination of Extractable Residues: Air-dried samples (100 g) of the incubated soil taken after 12 days, were placed in a paper thimble and extracted for 24 h in a Soxhlet extraction apparatus with 300 ml of methanol at a flow rate of 5 to 6 cycles per hour. Glass wool was added to cover the soil surface to help prevent the loss of soil particles. The extract was concentrated to about 10 ml with a rotary evaporator (Rotavapor RE-120, Büchi, Switzerland). Aliquots of each extract were analysed for their radioactivity.

Determination of Bound Residues: Extracted soil was air-dried to remove residual solvent and 0.5 g sub samples were subjected to combustion for residual bound radioactivity.

Organic Matter Fractionation: Twenty gram portions of soil incubated for 12 days were shaken with 200 ml of NaOH+Na_4 P_2O_7 (0.1N and 0.1M, respectively) solution for 1 hour and were

centrifuged and the supernatant (alkali extract) collected. Soil residue containing humin fraction was dried and analysed for ^{14}C. A portion of the alkali extract was acidified to pH 2.0 with H_2SO_4 followed by centrifugation. Residues containing humic acid (HA) were then dissolved in 0.1N NaOH; the supernatant contained fulvic acid (FA) fraction. The FA and HA solutions were analysed for ^{14}C as described below.

Determination of Radioactivity: Aliquots of extracts and dried soil samples were combusted in a Packard sample oxidizer (Model 306) to $^{14}CO_2$. The latter was absorbed in and mixed with appropriate volumes of Carbo-sorb and Permafluor V (Packard Instrument International, Switzerland). The radioactivity of the above samples was determined in a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3320). For $^{14}CO_2$ determination, 0.5 ml of alkali containing CO_2 was mixed with 1 ml distilled water and 10 ml Quickszint-212 (Koch-Light Laboratories Ltd.,FRG) in a scintillation vial and subjected to scintillation counting. The external standard technique was used to correct for quenching.

RESULTS AND DISCUSSION

The data presented in Fig. 1 indicate that ^{14}C-malathion is rapidly decomposed to $^{14}CO_2$. The loss of radiocarbon due to evolution of $^{14}CO_2$ during the incubation period of 12 days was 56%. It is clear that the rate of $^{14}CO_2$ evolution is initially very high. After 4 days the losses as $^{14}CO_2$ were 49% of applied radioactivity; then a slower but continual loss of $^{14}CO_2$ was observed upto 12 days. The 56% loss of $^{14}CO_2$ in 12 days indicates rapid mineralization of ^{14}C-malathion in soil.

Table 1 shows the results of the extraction of the residual ^{14}C-malathion in soil after 12 days. The Soxhlet extraction process was used for exhaustive solvent extraction. Methanol gave a higher extraction efficiency than acetonitrile, ethyl acetate, acetone, chloroform and benzene.

The distribution of ^{14}C-residues of labelled malathion in soil after 12 days of incubation showed that 56% of the applied ^{14}C-malathion was liberated as $^{14}CO_2$, 6% were extractable and 38% unextractable or bound residues. These bound residues of malathion constituted a significant proportion of the total insecticide residues in soil. Therefore, special attention needs to be given to their release and subsequent potential bioavailability to plant.

Table 2 gives the distribution of ^{14}C-activity in humic substances. After 12 days of incubation, the soil contained 44% of the added ^{14}C-malathion. Of the applied ^{14}C, 24.64% was recovered in HA+FA whereas 19.36% was in humin. The fulvic acid fraction contained 16.81% of the added activity amounting to 38.14% of the residual ^{14}C. Incorporation of ^{14}C in the humic acid fraction was only 7.83%. The incorporation of bound residues in soil humus has been also observed by other workers (1, 3, 6, 18, 21-25). Incorporation of ^{14}C in humus fractions may be direct or indirect. Indirect incorporation through microbial metabolism seems to be more probable since ^{14}C-malathion was found to be rapidly oxidized to CO_2 during incubation. It would be interesting to study the dynamics of labelled microbial biomass in soil incubated with malathion.

The presence of high amounts of ^{14}C in the fulvic acid fraction observed in this study is consistent with those reported recently by Khan for prometryn (22) and by Ambrosi et al. for phosalone(18). Fulvic acid bound residues may be of great importance with regard to their bioavailability since the FA fraction

is considered to be the dominant soluble organic fraction present in the soil solution under field conditions(22). Studies are, therefore, needed on the incorporation of pesticide residues in different organic matter fractions of soil and on the dynamics of these fractions in relation to the bioavailability of bound residues.

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Table 1. Comparison of extraction efficiency of different solvents for residual ^{14}C-malathion in soil after 12 days.

Extraction solvent	Extractable (% of residual-^{14}C)
Methanol	17.20
Acetonitrile	4.30
Ethyl acetate	3.62
Acetone	1.89
Chloroform	1.08
Benzene	0.80

Table 2. Distribution of ^{14}C from ^{14}C-labelled malathion in soil organic matter fractions.

Fraction	% of applied radioactivity	% of residual radioactivity
Alkali extractable	24.64	55.91
Humic acid	7.83	17.77
Fulvic acid	16.81	38.14
Humin	19.36	44.09

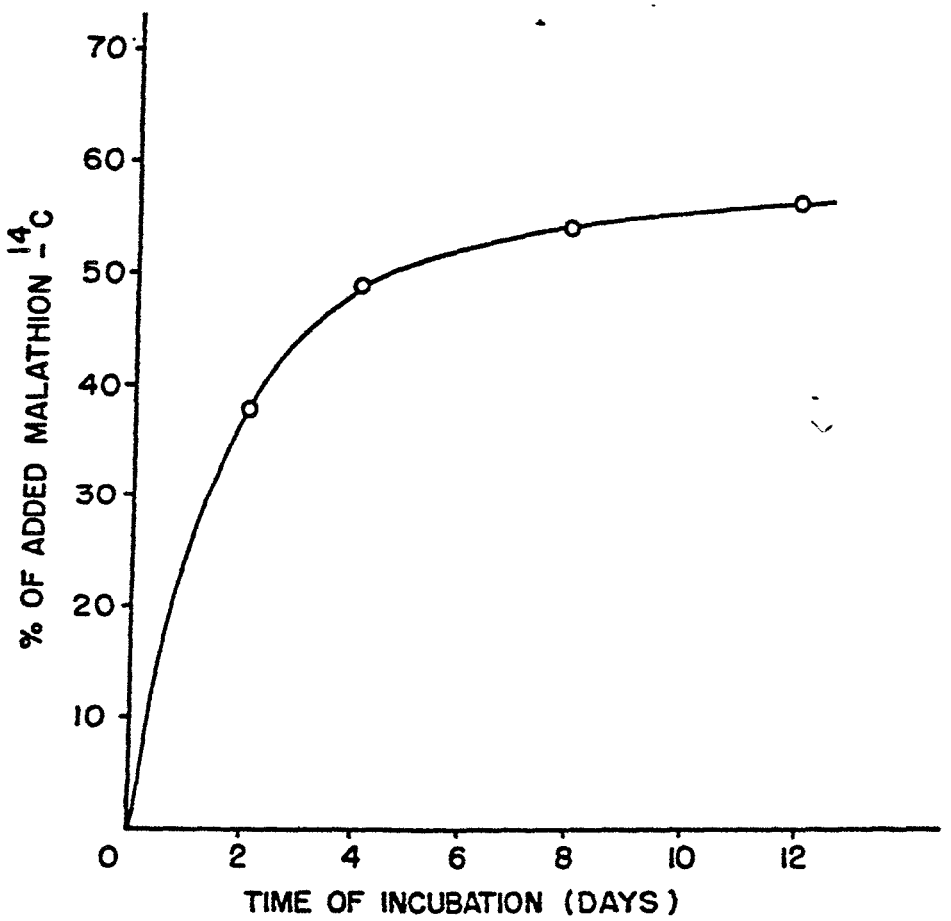

Fig. 1. Cumulative losses of $^{14}CO_2$ from soil incubated for 12 days with ^{14}C-malathion.

STUDIES OF ^{14}C-METHAMIDOPHOS RESIDUES AND THEIR BINDING TO COSTA RICAN VEGETABLES AND SOILS

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Abstract

Studies of ^{14}C-methamidophos residues in tomato, lettuce and two soils were made under greenhouse conditions. Residues in tomato fruits were 4.5 ppm 35 days after the last application. Total residues in lettuce were also high and present mainly in the edible leaves (9.7 and 12.8 ppm after 3 and 5 applications of the insecticide, respectively).

The non-extractable residues constituted a significant part of the total residues in plant tissues. Recovery of the extractable residues decreased with time more rapidly in a loamy clay than in a clay soil. Bound residue levels were about 8% of the applied ^{14}C in loamy clay after 10 days. Residues in the clay soil were higher and continued to decline throughout a 65-day period.

INTRODUCTION

Methamidophos is one of the most common insecticides used by Costa Rican farmers to protect many different crops, mainly vegetables.

In preliminary studies, we have found, that most farmers apply excessive doses and numbers of application of this insecticide.

Many farmers do not observe the recommended waiting period between the last application and harvesting.

The objective of the present study was the determination of the residues of methamidophos in tomatoes, lettuce and soils and their fate using ^{14}C-labelled insecticide.

MATERIALS AND METHODS

Tomato (Lycopersicum esculentum cv. Tropic) and lettuce seedlings (Lactuca sativa cv. White Boston) were cultivated using a loamy clay soil. Physical and chemical properties of this soil are pH = 5.0 (water); 6.2% organic matter; 31.2% clay and the cation exchange capacity 36.0 meq/ 100 g.

The ground soil was passed through a 10 mesh sieve; 4 liter plastic pots were used for growing the seedlings. Applications of the insecticide were made using a solution of ^{14}C-methoxy-methamidophos. The insecticide was supplied by Dr. Salah Sayed of the "Middle East Radioisotope Center", Cairo, Egypt. Specific activity was 3.4 mCi/g and radiological purity 99.9%, determined by TLC using three different solvent systems; 20.0 mg of the ^{14}C-insecticide and 176.7 mg of non labelled insecticide were dissolved in 250 ml water. Non labelled methamidophos was obtained from the Environmental Protection Agency , Research Triangle Park, NC, U.S.A., purity 99.9%.

Scintillation counting of 10 µl aliquots (Beckman, Model 8100-Spectrometer) showed a value of 3560.2 dpm. Efficiency was determined by the method of channel ratio using an external source.

A "Permablend" and naphthalene cocktail was used. Appropiated quench curves were determined. Total concentration of the insecticide amounted to 790 ppm in the applied solution. This concentration lies in the range of concentrations used by farmers and producers of tomato and lettuce in Costa Rica.

Table 1 shows the doses and periods between applications.

Besides this experiment a degradation study was started under greenhouse conditions. Soils at field capacity were spiked with 500.000 dpm each. Soil A corresponds to the soil used in the previous experiment. Soil B is a clay that showed following propierties: pH (water) 6.7; organic matter 2.1%; clay 58.2% and cation exchange capacity 62.0 meq/100 g.

Both soils were placed in plastic pots and the soil moisture was kept constant at field capacity during the experiment. Samples were collected at different periods of time.

For extraction of the methamidophos residues from plant samples, the method of Lubkowitz et al. (6) was used.

Recoveries using this method was excellent (99-101%). For scintillation counting a cocktail suitable for use with aqueous solutions was used. After extraction of the plant samples, they were dryed at room temperature and

thereafter extracted again in a soxhlet using methanol as a solvent during 24 hours, in order to assure exhaustive extraction. Efficiency of methanol extraction was in the range from 95-99%.

Soils were extracted with methanol for 40 hours in a soxhlet extractor. Each extract was concentrated at room temperature using a rotavapor.

Recovery tests of the insecticide from the soils, showed values between 54.3 and 66.5%.

After exhaustive extraction, samples of the soils and the plant material were ashed in order to determine bound residues. For this a Packard Tri-Carb Oxidyzer Model B-306 was used. Recoveries were in the order of 87%.

The distribution of the radioactivity among the extractable residues was studied using TLC techniques after Chevron Chemical Co. (2). The aqueous extracts were freeze dried and dissolved again in methanol. In all cases silica gel-G thin layer plates were used.

Results and Discussion

Analytical results of the ^{14}C-methamidophos residues and their distribution in leaves, stems, roots and fruits of the tomato plants, and the distribution in leaves and roots of the lettuce plants are given in Tables 2,3,4 and 5.

TLC experiments showed that almost all extractable activity comes from unchanged methamidophos.

Residues in tomato fruits are high despite the long period of time between the last application of the insecticide and harvesting. This may be due to the high doses used. Residues are also high in both green and old leaves. Smaller values were found in stems and roots. These findings are in agreement with the report of Bull (1) on the accumulation of the insecticide in tomato leaves after application.

Unpublished studies performed by the authors of this paper, using non labelled methamidophos in commercial crops, showed similar results. Other experiments using the recommended dose showed little residues 8 days afther the last application. Therefore the high doses commonly used by the farmers and also used in the present work, are responsible for the high levels of residues detected in this paper.

Total residues in lettuce were also high and present mainly in the edible leaves. Lower residues were found in stems and roots.

Residues found depend on the number of applications and the period of time between the last application and harvesting.

The non extractable residues were higher in stems and roots of the lettuce plants than in the leaves.

In all cases the extraction with water was very efficient. The second extraction using methanol removed only very small amounts of insecticide.

Residues in both crops exceeded the established tolerances for methamidophos, which ranged from 1 to 2 ppm for different vegetables (3,8).

TABLE 1. Applications of the ^{14}C-methamidophos solution on lettuce and tomato plants during the vegetative period.

Crop	Amount applied (ml)	Activity applied (uCi)	Time between applications (days)
Lettuce	0.5	0.13	--
Lettuce	3.5	0.91	22
Lettuce	3.0	0.78	16
Lettuce	5.0	1.30	16
Lettuce	6.0	2.08	18
Lettuce	6.5	1.64	22
Harvest	Σ = 26.5	Σ = 6.84	days between the last application and harvesting = 1
Lettuce	2.5	0.65	--
Lettuce	4.5	1.17	24
Lettuce	8.5	2.21	33
Harvest	Σ = 15.5	Σ = 4.03	days between the last application and harvesting = 3
Tomato*	0.5	0.13	--
Tomato	8.0	2.08	22
Tomato	8.5	2.21	16
Tomato	16.0	4.16	16
Tomato	12.0	3.12	18
Tomato	13.5	3.51	22
Tomato	17.5	4.55	14
Tomato	16.5	4.29	24
Tomato	21.0	5.46	24
Tomato	13.0	3.38	33
Tomato	14.5	3.77	22
Tomato	11.5	2.99	22
	Σ = 152.5	Σ = 39.65	

*Tomato fruits were sampled at different times during the vegetative period.

TABLE 2. Extractable and non-extractable ^{14}C-methamidophos residues in tomatoes

	Number of applications	^{14}C-methamidophos solution applied (ml)	Days between last application and harvesting	Extractable residues* (ppm)	Extractable residues** (ppm)	Non-extractable residues (ppm)	Total residues (ppm)
Fruits	7	76.0	8	4.24	0.28	0.40	4.92
Fruits	8	92.5	32	2.20	0.19	0.66	3.05
Fruits	9	113.5	29	3.67	0.20	0.43	4.30
Fruits	10	126.5	35	4.03	0.23	0.23	4.49
Fruits	12	152.5	40	0.07	----	----	0.07
Fresh leaves	12	152.5	40	14.70	0.20	4.69	19.59
Dead leaves	12	152.5	40	108.55	0.02	40.00	148.57
Stems	12	152.5	40	2.11	0.08	3.23	5.42
Roots	12	152.5	40	0.20	0.12	1.12	1.44
Soil	12	152.5	40	------	----	0.41	0.41
Leaching water	12	152.5	40	0.09	----	----	0.09

* Aqueous extraction
** Methanol soxhlet extraction

TABLE 3. Extractable and non-extractable ^{14}C-methamidophos residues in lettuce

Plant Part	Number of applications	^{14}C-methamidophos solution applied (ml)	Days between last application and harvesting	Extractable residues (ppm)	Extractable residues** (ppm)	Non-extractable residues (ppm)	Total residues (ppm)
Leaves	5	26.5	1	11.84	0.30	0.65	12.79
Stem and roots	5	26.5	1	0.83	0.11	0.93	1.87
Soil	5	26.5	1	-----	0.05	0.44	0.49
Leaching water	5	26.5	1	0.18	----	----	0.18
Leaves	3	15.5	3	9.00	0.25	0.49	9.74
Steam and roots	3	15.5	3	-----	0.12	0.29	0.41
Soil	3	15.5	3	-----	0.03	0.33	0.36
Leaching water	3	15.5	3	-----	----	----	-----

* Aqueous extraction
** Metanol soxhlet extraction

TABLE 4. Balance sheet for radioactivity in tomato

Plant Part	Extractable*	Extractable** dpm	Non extractable dpm	Total dpm
Fruits	568 925	36 033	68 863	673 821
Fresh leaves	283 426	3 856	90 426	377 708
Dead leaves	1259 209	232	464 010	1723 451
Stem	74 125	2 810	113 471	190 406
Roots	6 319	3 791	18 250	28 360
Soil	--------	-------	727 974	727 974
Leaching water	25 665	-------	-------	25 665
Applied total =	69479 000 dpm			Σ = 3 747 385 = 5.4% extractable and non-extractable activity

* Aqueous extraction

** Methanol soxhlet extraction

The recommended time period between the last application and harvesting varies from 14 to 21 days.

As a result we suggest the time period between the last application and harvesting should be increased in the case of tomatoes, if the farmers in Costa Rica continue applying the high doses used. Our results are in agreement with those reported by Lubkowitz et al. (6).

In the case of lettuce, harvesting was made 1 to 3 days after the last application in accordance with the usual practice followed by the farmers in Costa Rica. Therefore residues found were higher than the tolerances.

This results are in good agreement with unpublished studies by the authors using commercial crops and non-labelled methamidophos. In this case the doses were slightly higher and the residues found amounted to 15.07 ppm and 9.91 ppm, one and three days, respectively, after application. The analytical technique used was gas liquid chromatography (4,7). These results agree with those reported by Leidy (5) on the effect of dose and number of applications on residues of methamidophos.

TABLE 5. Balance sheet for radioactivity in lettuce

Plant Part	Extractable* dpm	Extractable** dpm	Non extractable dpm	Total dpm
Leaves	1032 750	26 167	56 697	1115 614
Stem and roots	7 234	959	8 105	16 298
Soil		86 405	789 235	875 641
Leaching water	25 655	------	-------	25 655
Applied total =	12073 400			Σ = 2033 208 = 16.8% extractable and non-extractable activity
Leaves	616 121	17 114	33 544	666 779
Stem and roots	---------	820	1 801	2 621
Soil	---------	56 974	595 720	652 695
Leaching water	---------	------	-------	-------
Applied total =	7061 800			Σ = 1322 095 = 18.7% extractable and non-extractable activity

* Aqueous extraction
** Methanol soxhlet extraction

In the case of both crops, some residues were also found in the soil and leaching water fractions, respectively.

In Tables 4 and 5 the balance of total activity shows a low recovery of the insecticide. The recovery is higher in lettuce than in tomato. This may due to a protective effect of the lettuce head, and size effect of the tomato plant.

Figure 1 and 2 shows the recovery of the insecticide from soils A and B. In case of soil A recovery of ^{14}C-methamidophos decreased rapidly with time. In the case of soil B, bound residues changed little with time. These findings are in agreement with other studies in soils (6).

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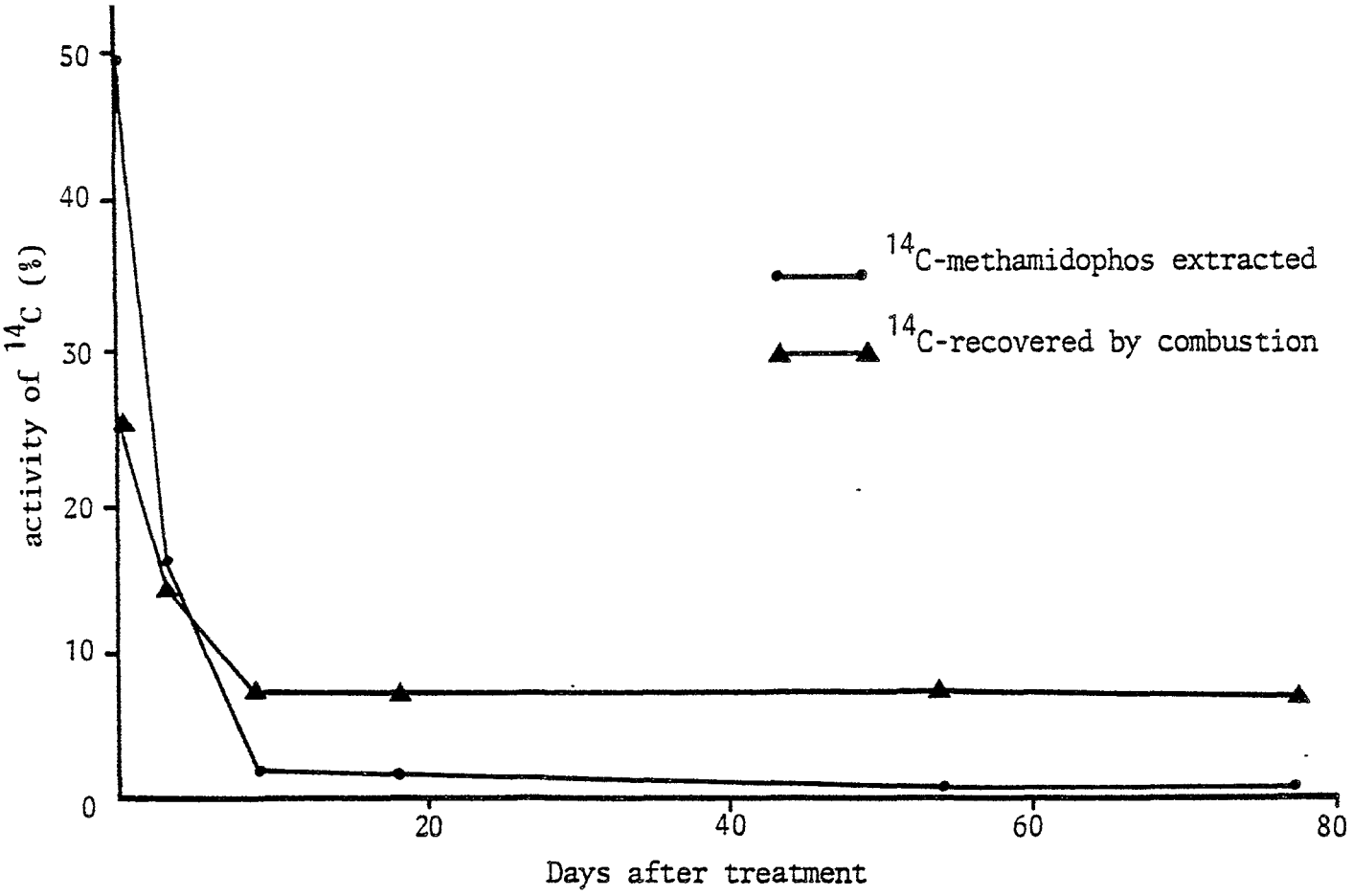

Fig. 1. Degradation of ^{14}C-methamidophos in soil A.

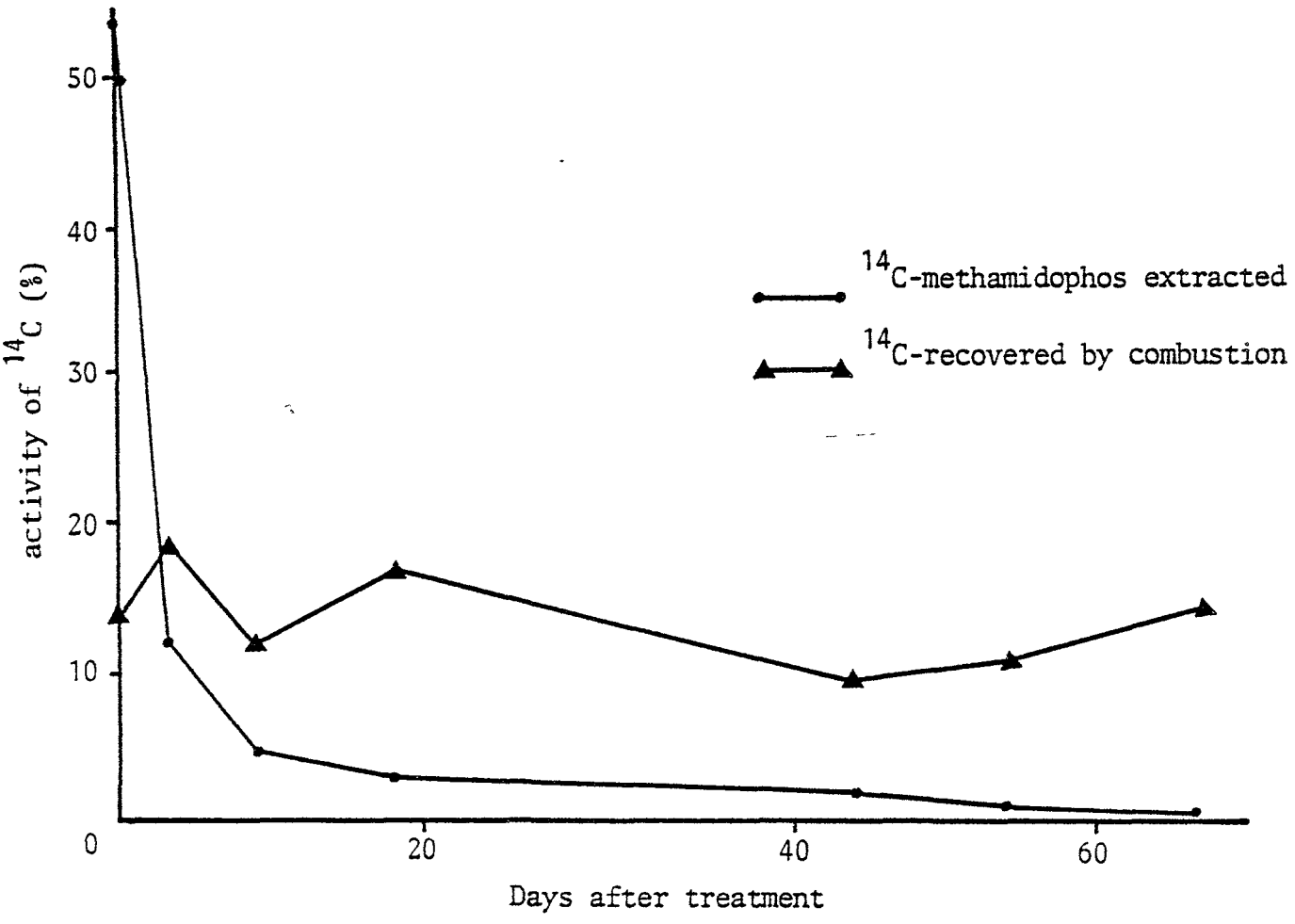

Fig. 2. Degradation of ^{14}C-methamidophos in soil B.

BOUND RESIDUES OF ^{14}C-CHLORFENVINPHOS IN WINTER RAPE AND SOIL

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Abstract

The binding properties of chlorfenvinphos were studied by application of ^{14}C-chlorfenvinphos to the leaves and stems of winter rape and two loamy sand soils of different organic matter content.

After application of vinyl-^{14}C chlorfenvinphos to the plants at a dosage of 0.3 mg/plant, no bound radioactivity was detected either in the flowers and siliques or in the roots. In the leaves and/or stems, the bound ^{14}C-residues, expressed as chlorfenvinphos, ranged from 0.02 µg/g one hour after the treatment to 0.18 µg/g at harvest. Repeated treatment applied to the plants 9 days after the first application caused no increase of the bound residue concentration. Various treatments of stem-bound ^{14}C with alkali and acid led to release of some radioactivity.

When vinyl-^{14}C chlorfenvinphos was applied to the soils, the bound ^{14}C-residues increased and the extractable ^{14}C-residues decreased with time, particularly in the soil of higher organic matter content. After 114 days, the bound ^{14}C-residues, expressed as percentage of the applied ^{14}C, amounted to 13.7% in the soil of higher and 11.2% in the soil of lower organic matter content. When soil was treated with ring-^{14}C chlorfenvinphos, the bound and extractable ^{14}C-residues were somewhat higher than those determined for vinyl label. Experiments suggested that binding was related to the activity of soil microorganisms since soil sterilization resulted in a reduction of bound ^{14}C-residues by 80% (vinyl label) and 79% (ring label).

INTRODUCTION

Chlorfenvinphos 2-chloro-1-(2',4'-dichlorophenyl)vinyl diethyl phosphate is an effective insecticide used both in soil and foliar applications. Studies of Beynon and Wright [1, 2,] and Beynon *et al.* [3] report on its fate in soil and plants. Using a radiolabelled insecticide, the authors demonstrated binding to various substrates.

When chlorfenvinphos proved to be effective as a foliar insecticide against pests attacking rape, a study of terminal residues in this plant was conducted by Dec et al. [4]. As the problem of bound pesticide residue formation has given concern to regulatory bodies, the present study was initiated to investigate formation of bound residues of ^{14}C-chlorfenvinphos in winter rape and soil.

MATERIALS AND METHODS

1. The radiolabelled insecticide

Radiotracer experiments with winter rape and soil were carried out using vinyl-1,2-^{14}C chlorfenvinphos (Z-isomer) of specific activity 2.65 µCi/mg. For soil experiments also ring-^{14}C chlorfenvinphos (Z-isomer) of specific activity 22.8 µCi/mg was applied. The radiolabelled insecticides were obtained from Shell Research Limited, Sittingbourne, Kent, UK.

2. Radiotracer experiments

a) Rape plants

The experiment was conducted on a plot of winter rape (variety Janpol), which was located in Winnagora near Poznan. At the full flowering stage, selected rape plants received one or two treatments with a 9-day interval of 2 ml water solution containing 0.3 mg of vinyl-^{14}C chlorfenvinphos. The solution was spread on the leaves and stems, using a little brush. Rape samples (two whole plants), taken after the treatments and at harvest, were separated into (i) roots, (ii) leaves and/or stems and (iii) flowers or siliques.

Extraction procedure: This was based on the method described by Beynon and Wright [1]. The plant segments were cut into small pieces, extracted twice with 30% acetone in hexane and filtered. The combined filtrates were washed with an equal volume of water to separate the acetone from the hexane layer. The aqueous acetone was evaporated until about 10 ml of water solution remained and then extracted twice with hexane. The hexane extracts were combined with the previous hexane layer.

The solids left after the 30% acetone/hexane extractions were extracted twice with absolute ethanol and filtered. Ten ml of water were added to the combined filtrates and the solution was concentrated until no more ethanol evaporated. The aqueous phase remaining was extracted twice with hexane. The hexane and water phases were pooled with those obtained previously and subjected to radiocounting. The solids were dried and subjected to oxygen combustion.

Fractionation of bound residues: The solids of stems sampled at harvest were additionally extracted twice with absolute ethanol and three times with water; the extracts being analysed for radioactivity. The extracted material (containing only bound residues) was dried, subjected to combustion and counted. To investigate the distribution of bound ^{14}C, the extracted stem solids were fractionated according to the method of Chmielewska [5]. Two hundred mg subsamples of the plant material were refluxed for 1 hr. with 0.7% KOH and centrifuged. The supernatant was concentrated to about 10 ml and precipitated with absolute ethanol. After filtration, both the filtrate and precipitate were examined for radioactivity. The sediments were subjected to further hydrolysis by shaking for 24 hr. with 17.5% NaOH. The mixture was centrifuged and the supernatant was precipitated with absolute ethanol and filtered. The filtrate and precipitate were analysed by radiocounting. The sedimented solids were further hydrolyzed by digestion with 40% HCl. After shaking for 48 hr., the mixture was diluted with water and filtered. The filtrate and the remaining solid were subjected to radiocounting.

b) Soil

Two loamy sand soils from Winnagora near Poznan were used in these studies. Some of the physical and chemical properties of these soils are summarized in Table I. The soils were partially air-dried at room temperature and worked through a 2 mm sieve.

Table I. Soil physical and chemical properties

	pH		Organic C	Sand	Silt	Floatable particles	Water holding capacity
	H_2O	KCl	%	%	%	%	g/100g
Soil 1	7.15	6.70	3.74	63	21	16	34.0
Soil 2	7.10	6.50	1.93	61	22	17	29.9

Laboratory experiments: Soil samples were placed in 50 ml Erlenmeyer flasks and treated with mixtures of labelled and unlabelled chlorfenvinphos. Soil moistures were adjusted to 60% of the maximum water holding capacity and maintained at that level throughout the experiment. The flasks were stoppered with cotton wool and incubated at about 23°C in the dark.

To examine the rate of bound residue formation in soils of different organic matter content, 21 g samples of soils 1 and 2 (19.0 g and 18.9 g oven-dry weight basis) were treated with 2 ml of aqueous solution containing 50 µg (0.13 µCi) of vinyl-^{14}C chlorfenvinphos and 50 µg of unlabelled chlorfenvinphos to give an insecticide concentration of about 5 µg/g. The samples were incubated for 0 (2 hr.), 7, 14, 28, 56 and 114 days.

In order to learn whether bound residues contain both vinyl and ring moieties of the chlorfenvinphos molecule, the same experiment was conducted with ring-^{14}C chlorfenvinphos, applying 2 ml of water solution containing 6 µg (0.13 µCi) of the radiochemical and 94 µg or 394 µg of unlabelled chlorfenvinphos to give final insecticide concentrations of about 5 and 20 µg/g. Soils were incubated for 0 (2 hr.), 7 and 28 days.

Both vinyl-^{14}C chlorfenvinphos (50 µg radiochemical + 50 µg unlabelled insecticide) and ring-^{14}C chlorfenvinphos (6 µg radiochemical + 94 µg unlabelled insecticide) were incubated in sterile soil 1 for 0 (2 hr.), 7 and 28 days to investigate the potential involvement of soil microorganisms in bound residue formation. To sterilize the soil, 500 g were placed in a 1000 ml jar, propylene oxide (2.0 ml) was added, and the jar was sealed for 2 days at room temperature. The sterilized soil was air-dried for one day and 20 g samples (19.7 g oven-dry weight) were transferred into 50 ml Erlenmeyer flasks and further sterilized by pasteurization for one hour on three successive days.

All experiments were carried out with three replicates.

Extraction procedure: At the end of the incubation period, the soil samples were extracted twice with 70 ml of methanol by shaking for 1 hour. After filtration, soil samples were Soxhlet-extracted with methanol for 17-20 hours. The combined extracts and the extracted soil were examined for radioactivity.

3. Radiocounting

Radiocounting was performed using an Intertechnique Model SL 30 liquid scintillation counter. Extracts and other solutions (2 ml aliquots) were counted in 10 ml of Bray's cocktail (8 g Butyl PBD + 60 g naphthalene + 100 ml methanol + 1000 ml dioxane). The radioactivity in plant materials and precipitates was determined by oxygen combustion. The evolved $^{14}CO_2$ was trapped in 10 ml of 12% ethanolamine in methanol and 2 ml aliquots were counted in 10 ml of Bray's cocktail with an addition of 0.6 g of Cabosil. The non-extracted residues in soil samples (2 g) were determined by the wet combustion method described by Smith et al. [6]. Quench corrections were made by external standard method, using a Multi 20 computer. For that purpose, adequate calibration curves were prepared and fed into the computer.

RESULTS AND DISCUSSION

Radioactive residues, found in samples of winter rape treated with vinyl-^{14}C chlorfenvinphos, are presented in Table II and Table III. It may be noted that no bound radioactivity was detected in the roots and flowers or siliques. In the leaves and/or stems, bound ^{14}C-residues (expressed as chlorfenvinphos) amounted to 0.02 µg/g (1.1% of the total residue) one hour after the first treatment and increased to 0.21 µg/g (28.4%) nine days later (Table II). At harvest, 0.18 µg/g (54.5%) was found. The second treatment caused no increase of the bound residue concentration (Table III).

The extractable residues were partitioned between hexane and water. Residues contained in the hexane phase (non-polar compounds) decreased with time. Residues contained in the aqueous fraction also decreased but their contribution to the total residue increased (values in parentheses in Table II and Table III).

The solids of stems sampled at harvest containing 0.18 µg/g of unextracted residue were additionally extracted with absolute ethanol and then with water. After extraction, 0.10 µg/g was still present.

To investigate the distribution of bound ^{14}C, the extracted stem solids were fractionated by subsequent treatments with 0.7% KOH, 17.5% NaOH and 40% HCl. After KOH treatment followed by precipitation with ethanol, 0.04 µg/g was found in the filtrate and 0.01 µg/g in the precipitate. The NaOH hydrolysis followed by precipitation with ethanol released 0.01 µg/g found in the precipitate. In the filtrate obtained after the HCl digestion, no radioactivity was detected while in the lignin remnant 0.04 µg/g was found. This constituted 12.1% of the total residue present in the stems at harvest.

The rates of bound residue formation in soils 1 and 2, treated with vinyl-^{14}C chlorfenvinphos, are shown in Figure 1. The amount of bound ^{14}C-residues in soil 1 was slightly higher than in soil 2. Two hours after soil treatment, 0.6 $\pm$ 0.1% of the applied radiocarbon was bound to soil 1 and none was bound to soil 2. After 28 days, bound ^{14}C-residues increased to 18.0 $\pm$ 1.1% and 12.5 $\pm$ 0.3%, respectively. After 56 days, there was a decrease to 14.1 $\pm$ 0.4% and 11.9 $\pm$ 1.7%, respectively. This level remained practically constant throughout the rest of the experiment. The decrease of bound ^{14}C-residues between 28 and 56 days may be related to rapid growth of soil fungi stimulated by favourable incubation conditions. Five or six weeks after insecticide application, the fungi became visible as a mould on the soil surface. The mould was cultivated in a potato-glucose agar medium and identified as Botrytis sp.

When samples of soil 1 were treated with ring-^{14}C chlorfenvinphos (5 µg/g), both bound and extractable ^{14}C-residues were somewhat higher than those determined for vinyl-^{14}C chlorfenvinphos (Figure 2). This may suggest cleavage of the chlorfenvinphos molecule. However, it must be noted that the experiments were not conducted simultaneously and the soil moisture differed significantly (60% in case of vinyl label and 80% in case of ring label) in the early stages of the incubation period. The application of a higher concentration (20 µg/g) resulted in a higher rate of bound ^{14}C-residue formation and slower decrease of extractable ^{14}C-residues.

The results of the experiments with sterile soil 1 (Figure 3) indicate that binding of ^{14}C-residues was related to the activity of soil microorganisms. Sterilization of the soil did not eliminate the binding, however, bound ^{14}C-residues in the sterile soil (28 days after application) were reduced by 80% (vinyl label) and 79% (ring label) as compared to those determined in non-sterile soil. The decline of extractable ^{14}C-residues in sterile soil was insignificant.

ACKNOWLEDGEMENT

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Table II. ^{14}C-residues in rape samples after one treatment with vinyl-^{14}C chlorfenvinphos

Plant material	Interval after treatment (days)	Residue µg/g			
		Hexane	Water	Bound	Total
Flowers	0	< 0.01	< 0.01	< 0.01	< 0.01
or	9	< 0.01	0.01	< 0.01	0.01
siliques	62*	< 0.01	0.01	< 0.01	0.01
Leaves	0	1.81 (96.7)	0.04 (2.2)	0.02 (1.1)	1.87 (100.0)
and/or	9	0.33 (44.6)	0.20 (27.0)	0.21 (28.4)	0.74 (100.0)
stems	62*	0.03 (9.1)	0.12 (36.4)	0.18 (54.5)	0.33 (100.0)
	0	< 0.01	< 0.01	< 0.01	< 0.01
Roots	9	0.01	0.01	< 0.01	0.02
	62*	0.01	0.02	< 0.01	0.03

*Harvest time (29 July).

Values in parentheses refer to percent of the total residue.

Table III. ^{14}C-residues in rape samples after two treatments with vinyl-^{14}C chlorfenvinphos

Plant material	Interval after the second treatment (days)	Residue µg/g			
		Hexane	Water	Bound	Total
Flowers	0	0.04	0.02	< 0.01	0.06
or	32	< 0.01	0.02	< 0.01	0.02
siliques	53*	< 0.01	0.01	< 0.01	0.01
Leaves	0	3.84 (87.3)	0.44 (10.0)	0.12 (2.7)	4.4 (100.0)
and/or	32	0.08 (15.4)	0.26 (50.0)	0.18 (34.6)	0.52 (100.0)
stems	53*	0.04 (11.7)	0.17 (50.0)	0.13 (38.3)	0.34 (100.0)
	0	0.04	0.01	< 0.01	0.05
Roots	32	0.02	0.03	< 0.01	0.05
	53*	0.01	0.02	< 0.01	0.03

0 time = nine days after the first treatment.

*Harvest time (29 July).

Values in parentheses refer to percent of the total residue.

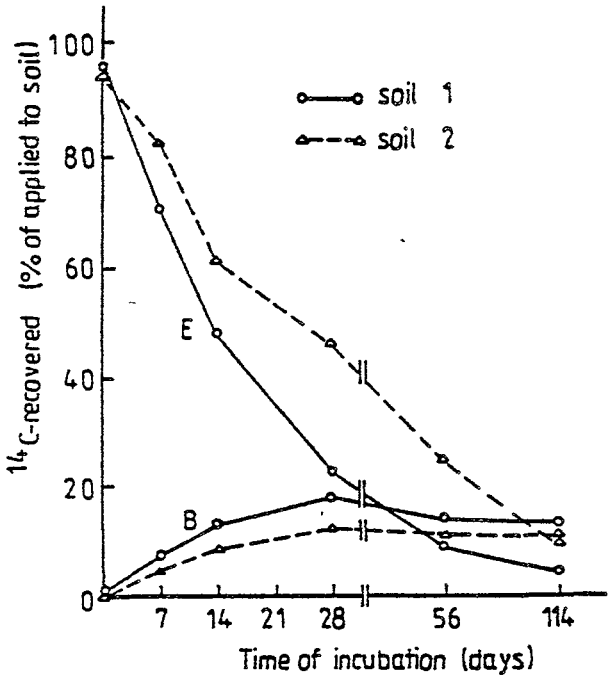

Fig. 1. Bound and extractable radioactivity in soil 1 and soil 2 treated with vinyl-^{14}C chlorfenvinphos /5 µg/g.
E = extracted-^{14}C, B=bound-^{14}C.

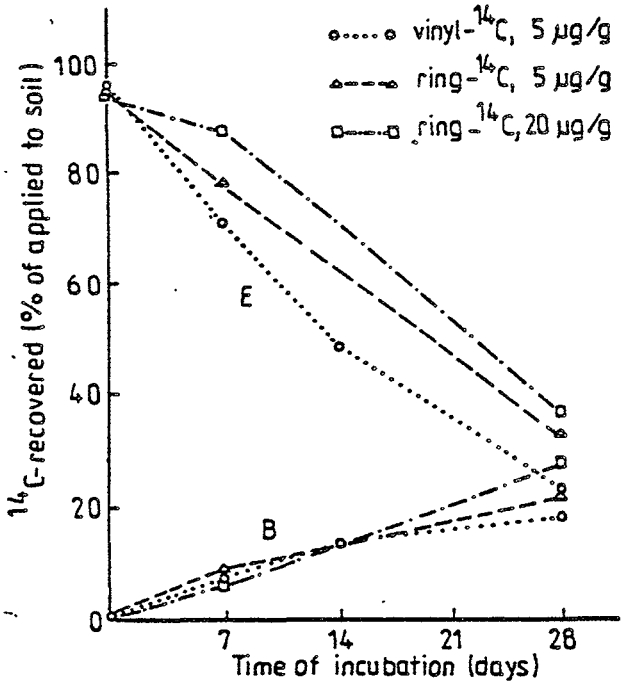

Fig. 2. Bound and extractable radioactivity in soil 1 treated with ring-^{14}C chlorfenvinphos. For comparison, data are inserted for bound and extractable residues of vinyl-^{14}C chlorfenvinphos in soil 1. E = extracted-^{14}C, B=bound-^{14}C.

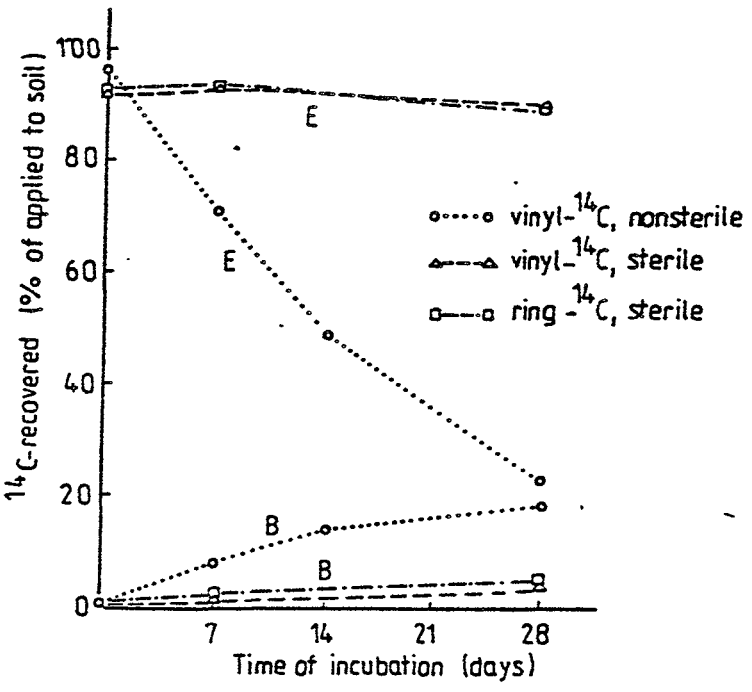

Fig. 3. Bound and extractable radioactivity in sterilized soil treated with both vinyl- and ring-labeled chlorfenvinphos (5 µg/g). For comparison, data are inserted for bound and extractable residues of vinyl-^{14}C chlorfenvinphos in non-sterile soil. E = extracted-^{14}C, B = bound-^{14}C.

BOUND RESIDUES OF ISOPROCARB IN THE COMPONENTS OF THE RICE ECOSYSTEM

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Abstract

Aspects relating to the binding of isoprocarb in paddy soil were investigated. Stirring did not affect the loss of pesticide from water. Extractable isoprocarb in soil increased and levelled off at 10 to 15 days, decreasing thereafter. The distribution coefficient of isoprocarb in soil and water further supports the possibility of bound residue formation.

INTRODUCTION

The fate of isoprocarb (2-isopropylphenyl N-methyl carbamate) in the rice paddy environment was studied by Bajet and Magallona [1] who found that the ultimate sink of isoprocarb is the soil. The rate of soil-bound residue formation as well as desorption and bioavailability is important in relation to potential environmental significance.

MATERIALS AND METHODS

^{14}C-isoprocarb labelled at the isopropyl group (specific activity: 7.1 mCi/mmole, radiochemical purity more than 99%) was obtained from Mitsubishi Chemicals Industries. A stock solution of 0.62 µCi/ml of radiolabelled isoprocarb in methanol was prepared.

Beckman Ready Solv liquid scintillation cocktail was used and radioactivity was determined using a Packard liquid scintillation counter.

Rate of soil-bound residue formation

Two kg of paddy soil were placed in a shallow pan. Approx. 2 l tap water were added to give a depth of 2 inches to simulate field conditions. Five ml radiolabelled isoprocarb solution were added to the paddy water and the pan was covered to minimize volatilization.

Paddy water samples were collected after 0, 1, 2, 3, 6, and 10 days and then at 10-day intervals up to 5 months. Another set of paddy water samples was collected after disturbance of soil sediments by stirring for 1 min. The soil particles were filtered off and radioactivity of the paddy water was directly determined by LSC.

An equivalent set of soil samples (approximately 50 g) was collected at the same intervals up to 5 months. The paddy soil was centrifuged for 20 min. and the liquid was discarded. Twenty grams soil were analysed for extractable residues by a 20 hr. Soxhlet extraction with methanol. The amount of sample was increased as the amount of extractable residues decreased. The extract was evaporated almost to dryness by a rotary evaporator and the residue was taken up in 5 ml methanol. Radioactivity of the extract was determined by LSC.

The extracted soil was analysed for bound residues by high temperature distillation (HTD) [2, 3]. The distribution coefficient Kd was calculated using the following formula:

$$Kd = \frac{\frac{\mu \text{ moles}}{\text{kg}} \text{ absorbed in the soil}}{\frac{\mu \text{ moles}}{\text{litre}} \text{ in paddy water}}$$

Desorption of absorbed/adsorbed isoprocarb in soil

Approximately 1-2 g paddy soil were weighed into a vial and 10 ml of 0.01 M $CaCl_2$ were added. The mixture was agitated by the use of a wrist action shaker for 8 hr. The mixture was centrifuged for 20 min. and the water was filtered into another vial. The soil was desorbed with another 10 ml of 0.01 M $CaCl_2$ and the pooled extract was directly analysed for radioactivity by LSC.

As the amount of desorbed residue decreased, the pooled water extract was extracted with 50 ml of 1:1 petroleum ether:methylene chloride and re-extracted twice with 50 ml methylene chloride. The pooled solvent extracts were evaporated in a rotary evaporator and residues were taken up in 3 ml methanol.

RESULTS AND DISCUSSIONS

Stirring was done to evaluate the effect of disturbance on the concentration of isoprocarb in paddy water. Decrease in the concentration after stirring may mean adsorption by soil particles but an increase may be due to desorption from the soil. No general trend, however, was observed (Table 1).

In paddy soil, there was an increase in extractable residues up to 10 days (Table 1). However, after 21 days a decreasing trend was observed which may indicate the formation of bound residues in the soil. The amount of isoprocarb remaining in paddy water is given in Table 2.

An increasing distribution coefficient (ratio between extractable soil residue and paddy water residue) up to 32 days indicates the transfer of residue from the paddy water to the soil (Table 3). After 40 days, the distribution coefficient decreased.

The amount of isoprocarb desorbed per gram soil increased up to 2 days and started to decrease after 3 days (Table 4). Low desorption up to 1 day was due to low adsorbed isoprocarb. Table 4 shows that most of the extractable residues are desorbed easily up to 3 days. Desorption started to decrease thereafter up to 10 days. After 21 days, most of the extractable residues in the soil could not be easily desorbed by water.

Work is in progress to investigate the bioavailability of soil-bound isoprocarb residues to rice plant. Extractable residues will be removed from soil by methanol Soxhlet extraction.

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Table 1

Distribution of isoprocarb in flooded paddy soil

Time(days)	Concentration Paddy Water without stirring	Paddy Water with stirring	Paddy Soil Extractable Residues Soxhlet
BACKGROUND	-	-	0.5 dpm/g .0061 ppb
0	4161 dpm/ml 0.0507 ppm	3606 dpm/ml 0.0439 ppm	249 dpm/g 3.0362 ppb
1	3049 dpm/ml 0.0371 ppm	3072 dpm/ml 0.0374 ppm	496 dpm/g 6.0487 ppb
2	2665 dpm/ml 0.0325 ppm	2667 dpm/ml 0.0325 ppm	716 dpm/g 8.7232 ppb
3	2379 dpm/ml 0.0300 ppm	1672 dpm/ml 0.0204 ppm	643 dpm/g 7.8401 ppb
6	1843 dpm/ml 0.0224 ppm	1508 dpm/ml 0.0184 ppm	765 dpm/g 9.3148 ppb
10	1172 dpm/ml 0.0143 ppm	1457 dpm/ml 0.0177 ppm	776 dpm/g 9.4526 ppb
21	960 dpm/ml 0.0117 ppm	435 dpm/ml 5.2988 ppb	653 dpm/ml 7.9543 ppb
32	230 dpm/ml 2.8016 ppb	244 dpm/ml 2.9722 ppb	381 dpm/g 4.6410 ppb
40	176 dpm/ml 2.1438 ppb	287 dpm/ml 3.496 ppb	260 dpm/g 3.1671 ppb
54	185 dpm/ml 2.2535 ppb	253 dpm/ml 3.0818 ppb	204 dpm/g 2.4849 ppb
60	220 dpm/ml 2.6798 ppb	160 dpm/ml 1.9489 ppb	132 dpm/g 1.6079 ppb
150	198 dpm/ml 2.4118 ppb		43 dpm/g 0.5237

Table 2

Isoprocarb in paddy water (% of initial concentration)

Time(days)	without stirring	with stirring
1	73.28	85.19
2	64.05	73.96
3	57.17	46.37
6	44.29	41.82
10	28.17	40.40
21	23.07	12.06
32	5.53	6.77
40	4.23	7.96
54	4.45	7.02
60	5.29	4.44
150	4.76	

Table 3

Distribution coefficient of isoprocarb in soil and water

Time (days)	Kd
0	.0599
1	.1630
2	.2699
3	.2634
6	.4191
21	0.6802
32	1.6565
40	1.4773
54	1.1027
60	0.6000
150	0.2172

Table 4

Desorption of absorbed/adsorbed isoprocarb residue in paddy soil

Time(Days)	Concentration desorbed per gram soil (ppb)	% desorbed in relation to extractable residues
0	2.6433	87.06
1	5.8592	96.37
2	8.3928	96.21
3	8.0030	100
6	7.3000	78.37
10	7.0285	74.36
21	1.0150	12.76
32	0.6317	13.61
40	0.3038	9.59
54	0.1827	7.35
60	0.0873	5.43

POSSIBLE UPTAKE OF METHOMYL BY BEAN PLANTS

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Abstract

The bioavailability to bean plants of ^{14}C-labelled methomyl applied to Egyptian soil has been investigated, using four different application rates. The amounts of radioactivity in bean plants were found to depend on pesticide concentration as well as on the time of plant growth. In general, the amounts detected in roots were significantly higher than those found in shoots.

INTRODUCTION

The pesticide methomyl (Lannate), S-methyl N-(methoxycarbamoyl)oxy thioacetimidate, has shown good results for the control of the Egyptian cotton leaf worm Spodoptera littoralis (Boisd) [1] and cabbage aphids [2]. Its residues on and in some vegetables have recently been investigated [1, 3]. Following topical application, the residual effect of methomyl in squash, snap beans, tomatoes, eggplant and okra fruits was found to be short. The safe waiting period between application of the pesticide and marketing the edible part of the treated crop was found to range from 1 day for squash and eggplant fruits, 3 days for okra fruits, to 6 days for both snap beans and tomatoes [1]. Similar results were obtained with corn leaves where a decline in methomyl residues with a half-life value of 2.5 days was calculated [3].

The present investigation was conducted to assess the possible uptake of ^{14}C-labelled methomyl through the root system of dicot plants from Egyptian soil. This is an essential preliminary study for the elucidation of the probable toxicity of plant-bound residues of methomyl to mice.

MATERIALS AND METHODS

Soil samples (1 kg each) were placed in 10 cm diameter pots. Methomyl (S-methyl-^{14}C)* (sp. act. = 2 μCi/mg) was thoroughly mixed with the soils to give concentrations of 2, 4, 6 and 12 ppm, corresponding with 2.3, 4.6, 6.9 and 13.8 μCi, respectively. Bean seeds (Vicia faba) were sown in the pots and left to grow under normal growth conditions. Plant samples were harvested after 2, 4 and 6 weeks by cutting the shoots approximately 1 cm above soil surface. The root systems were carefully removed and rinsed with tap water. Samples (50 mg) of the dried plant tissues were combusted in a Packard sample oxidizer (306), and the ^{14}C measured by counting in an LSC. Samples were analysed in duplicates and average values are reported. The residue levels are indicated on dry weight basis.

RESULTS

The amounts of ^{14}C-labelled methomyl detected in shoots and roots of bean plants are illustrated in Table I. It can be seen that these amounts increased with increasing concentration of the pesticide and the time of growth. Larger amounts of radioactivity were found in the root tissues than in shoot tissues especially at higher levels of pesticide contamination. Referring to the safe level of tolerance established in 1971 by EPA for methomyl (0.2 ppm) in or on food, the amounts detected in the shoot systems in the present study are below that level up to a soil concentration of 4 ppm which corresponds to 20 times of that applied in the field. Compared with the controls, no obvious morphological deformations of any kind or retardation in growth were noticeable with all concentrations used. This indicates that methomyl does not possess any significant phytotoxic properties.

For the study of the possible biological activity of bound residues of methomyl in mice, an experiment has started in which carrot plants have been sown in pretreated soils. Determination of the residual levels in the roots, extraction of tissues and feeding to mice is expected to be carried out at the end of this summer.

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*Supplied by courtesy of Prof. Willis B. Wheeler.

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Table I: Concentrations (ppm) of ^{14}C-labelled methomyl detected in bean plants

Methomyl concentration in soil (ppm)	Root			Shoot		
	Growth period (weeks)					
	2	4	6	2	4	6
2	0.08	0.09	0.11	0.08	0.08	0.10
4	0.12	0.15	0.18	0.10	0.12	0.14
6	0.24	0.30	0.34	0.17	0.20	0.22
12	0.38	0.43	0.71	0.21	0.25	0.32

PERSISTENCE OF PARAQUAT IN THE SOIL AND OBSERVATIONS WITH OTHER HERBICIDES RELEVANT TO THE THEME OF BOUND RESIDUES

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Abstract

Results from three separate experiments that have some relevance to bound residues are reported. In the first, ^{14}C-labelled paraquat was lost when applied to soil in the field, about 26% of the radioactivity disappearing in 15 months, whereas in laboratory incubation studies there was no loss of radioactivity in one year. Two possible explanations are (i) that there was photolytic decomposition in the field, (ii) the preparation of the soil for the laboratory study upset the microbial ecology of the soil to the detriment of organisms that can degrade paraquat.

In an experiment with ^{14}C-labelled isoproturon, there was an indication that there was slightly more ^{14}C in the unextractable humin fraction in soil in which wheat plants were grown than in bare soil.

Work in the UK, Federal Republic of Germany and in Switzerland has shown that the phytotoxicity of residues of atrazine, carbetamide, chloridazone, propyzamide, simazine, lenacil, monolinuron, linuron, propachlor and methabenzthiazuron can be satisfactorily predicted on the basis of the amount that is extractable with water. This implies that bound residues of these compounds, if they exist, are unlikely to be phytotoxic.

INTRODUCCION

Although the work with paraquat does not involve a bound residue as defined at the First Coordination Meeting in Costa Rica, the results recently obtained may have implications in relation to the methodology of conducting bound residue investigations. Similarly other work on the fate and availability of herbicides in soils, though not designed to study bound residues, is of interest in relation to assessing the likely occurrence and importance of bound residues for particular compounds.

a) Persistence of paraquat

In the report to the First Coordination Meeting the apparent loss of paraquat from field plots contrasted with the results of a laboratory incubation experiment with ^{14}C-labelled paraquat in which after 12 months only 0.5% of the applied radioactivity appeared as volatile products and the remainder, within experimental error, could all be extracted as paraquat.

The field plots have been maintained and have since been used for studies with ^{14}C-labelled paraquat in the field.

Materials and methods

Three replicate field plots were used that receive single annual applications of 4.48 kg ha^{-1} paraquat and in which natural vegetation is allowed to establish (Treatment Pq4, see Working Paper No. 7 of the meeting in Costa Rica or Fryer et al., 1975). Sections of plastic drainpipe, 10 cm diameter and 15 cm long were driven into the plots, 6 in one plot and 7 in each of the others. The soil surface in each of the tubes was treated in September 1981 with 2.5 µCi of ^{14}C (methyl) paraquat (sp. act. 43 mCi/mM) applied in 5 ml water with a pipette.

Two pipes with their contents were removed on the day of treatment and thereafter at 90-day intervals pairs of pipes were excavated. The soil was removed from each pipe, weighed and sieved through a 2.5 mm sieve into a

polyethylene bag. The bag was inflated and then shaken in order to mix the soil thoroughly. Duplicate subsamples (10 g) were taken for moisture determination and triplicate 1 g samples (air dry basis) were burnt in a Harvey Oxidiser and the evolved CO_2 trapped in 15 ml Reich's solution (7 g 2-(4'-t-butylphenyl)-5-(4"-biphenyl) 1,3,4-oxadiazole, 200 ml methanol, 330 ml phenethylamine and 400 ml toluene made up to 1 litre with water). Samples were counted on a Packard Tri-carb 3255 liquid scintillation spectrometer using an external standard.

Results and Discussion

Table 1 shows the results from the first 6 sampling dates.

After 15 months about 26% of the applied radioactivity has apparently disappeared. This rate of loss is higher than the average rate of loss of unlabelled paraquat from these plots which has been about 10% over the 14 years of the experiment. However, given the variability unavoidable in field data, the agreement is reasonably satisfactory.

Table 1. Radioactivity present in field samples treated with ^{14}C paraquat

	Time after application (months)					
	0	3	6	9	12	15
dpm x 10^{-6}	5.73	5.12	4.81	-	4.44	4.24
SE x 10^{-6}	0.16	0.15	0.24	-	0.16	0.12

The loss of radioactivity provides good evidence that paraquat is being degraded in the field so that the reduction in the percentage of extractable unlabelled paraquat observed is unlikely to be the result of changes in extractability. However, the question remains of why there was no loss of radioactive paraquat when incubated with soil from the same plots in the laboratory.

There are a number of possible explanations. One is that the losses in the field were due to wind erosion of the surface soil. This is thought to be unlikely since the plots containing the radioactive treatments were covered with vegetation for most of the period. In addition the rate of loss of unlabelled paraquat over the years has been the same from plots kept weed free as those in which weeds have been allowed to grow. A second possibility is that the loss in the field is caused by photolytic reactions which would have been precluded in the laboratory. This is possible since paraquat adsorbed on soil surfaces can be degraded by ultra-violet light and paraquat on leaf surfaces is susceptible to sunlight (Summers, 1980). However, since a measurable loss of radioactivity occurred in the period September-March when light intensities are low in England, this explanation seems unlikely. The third possibility is that bringing soil into the laboratory and preparing it by sieving upset the microbial ecology of the soil to the detriment of organisms that can degrade paraquat. The usual manipulations involved are known to affect the biological properties of soil in general (Greaves & Malkomes, 1980). In addition Hance & Haynes (1980) found differences in the rate at which linuron and metribuzin were degraded in the laboratory between soil kept in bags and undisturbed soil cores. This is therefore a plausible explanation, except that it is usually possible to predict field persistence of pesticides approximately on the basis of degradation rates observed in the laboratory (Hurle & Walker, 1980; Laskowski et al., 1983). Paraquat is an unusual compound in this context because of its adsorption characteristics so a more specialised microflora may be needed to degrade it than is generally the case. Thus small perturbations of the soil could upset the balance sufficiently to stop paraquat degradation. Although this discussion is entirely speculative, it supports the view that with present knowledge it is unwise to extrapolate from laboratory to field without at least some confirmatory field data.

This programme will continue by attempting to check on the possibilities outlined above.

b) Recent observations with other herbicides

Decomposition of isoproturon in soil.

The object of the work was to compare the decomposition of isoproturon in soil containing growing plants with that in soil without plants.

Materials and methods

The soil was a sandy loam containing 14% clay, 16% silt, 38% fine sand, 32% coarse sand and 1.7% organic carbon. The pH of a 1:2 soil/water slurry was 7.6, the cation exchange capacity was 22 meq/100 g, and the water holding capacity was 16.6%.

Soil treatment. Radioactive isoproturon, ^{14}C-isopropyl labelled, specific activity 20.16 $\mu Ci\ mg^{-1}$, donated by CIBA-GEIGY AG, Basle, was dissolved in methanol (2.45 mg ml^{-1}). The methanol solution was diluted with water (1:49). An aliquot of this solution (50 ml) was applied by pipette to 2.4 kg (oven dry basis) of moist soil (10% water) spread in a layer about 25 mm deep). The soil was thoroughly mixed by shaking in a plastic bag and then 250 g samples were transferred to 76 mm diameter plastic pots. Half the pots were sown with 5 seeds of wheat. The pots were placed in a glasshouse where the temperature ranged from 12-16°C and lighting was used to give a 14 h day at a minimum light intensity of 50 w/m^2. Seeds were thinned to 3 per pot as before. Triplicate pots were taken for analysis after 2, 4 and 6 weeks.

Extraction. Sub-samples (25 g) of soil were sequentially extracted according to a modification of the scheme of Hsu and Bartha (1976) as follows:-

a) The soil was shaken for 1 h with 50 ml methanol on a wrist action shaker. The slurry was centrifuged at 2100 g for 10 min and the supernatant liquid decanted.

b) The soil residue was shaken for 1 h each with successive portions of 50 ml and 25 ml acetone as in (a).

c) The soil residue was refluxed with 100 ml NaOH (500 g l^{-1}) for 3 h. The resulting suspension was steam distilled for 0.5 h and the distillate condensed in 10 ml 0.1 M HCl. The slurry was then centrifuged at 2100 g for 20 min and the supernatant liquid decanted. The residue was shaken for 5 min with 25 ml distilled water, centrifuged as before and the water added to the NaOH extract. The washing procedure was repeated 4 times. The soil residue was dried at 105°C for 24 h.

d) The alkaline extract was acidified to pH 1 with conc HCl and the humic acid suspension centrifuged at 2100 g for 10 min. The precipitate was dried at 105°C for 24 h. The fulvic acid solution was evaporated to dryness on a boiling water bath.

Estimation of ^{14}C. Portions (1 ml) of the methanol, each of the two acetone extracts and the distillate were added directly in a glass counting vial to 10 ml of a mixture of 5.5 g PPO (2,5-diphenyloxazole) and 0.1 g POPOP (1,4-di-2-(4-methyl-5-phenyloxazolyl) benzene) dissolved in 333 ml Triton X-100 and made up to 1 l with toluene. Samples were counted on a Packard Tri-carb 3255 liquid scintillation spectrometer using an external standard.

The solid components (soil residue, humic acid and fulvic acid) were separately wet oxidised essentially by the method of Allison et al. (1965). The traps between the reaction flask and absorbent flask contained 50% aqueous KI to remove Cl_2, conc H_2SO_4, a Zn metal column to remove acid fumes anhydrous $MgClO_4$ to remove water. The CO_2 was absorbed in 10 ml Reich's solution and the radioactivity counted as before.

Estimation of organic carbon. Total carbon in soil and the various extracts was determined by the method described by Greaves et al. (1978) which is an automated dichromate oxidation method.

Results and discussion

The distribution of ^{14}C in the various fractions obtained at 6 weeks is summarised in Table 2.

Table 2. Distribution of radioactivity at 6 weeks in soil treated with ^{14}C isoproturon in the absence and presence of wheat plants

	Radioactivity (% total applied)			
	Bare soil		Soil with plants	
Methanol	60.6	(5.6)	62.5	(4.8)
Acetone I	16.5	(1.1)	16.1	(2.2)
Acetone II	5.3	(0.6)	5.0	(0.5)
Distillate	7.8	(0.7)	5.2	(1.0)
Humic acid	4.0	(1.7)	4.8	(1.2)
Pulvic acid	0.8	(0.3)	1.3	(0.6)
Residue	5.34	(0.9)	7.9	(1.3)

The values are the mean of 3 replicates with standard errors in parenthesis.

Over 80% of radioactivity was extracted with methanol or acetone. Of the remainder, more than half was released by alkaline hydrolysis and steam distillation but an unextractable residue of 5-8% remained. This is of the same order as that found by Bollag et al. (1978) after incubating 4 substituted anilines in soil for 6 weeks (8-18%) and Hsu & Bartha (1976) with 3,4-dichloroaniline (9.1% after 9 weeks). Although not significant in the strict statistical sense, it is interesting that the distillable fraction was higher in the bare soil than the planted soil whereas the residue was higher in the planted soil. This suggests that there may be qualitative or quantitative differences in the breakdown in planted compared with unplanted soil. Intuitively this might be expected since the microbial population of the rhizosphere is more active and has a difference species composition to that in

root-free soil. However, the analysis of the methanol and acetone extracts showed that the same products were present in both soil systems. Also in a parallel kinetic study the rate of disappearance of parent isoproturon was not affected by the presence of wheat plants, the time to 50% loss being about 15 days at 15% water content and 20°C.

This work, a full account of which will be found in Mudd et al. (1983), was not designed to study bound residues but it does provide some evidence to suggest that studies of the role of rhizosphere organisms in incorporating residues containing ^{14}C into unextractable components might be worthwhile.

c) The use of water extractable residues to estimate residual phytotoxicity

If bound residues of herbicides are to have agricultural significance, then one way in which this could be manifest would be as unpredictably high phytotoxicity. For this reason work in Germany, Switzerland and at WRO to assess the availability of residues may be of interest.

The basic experimental details can be found in Pestemer et al. (1980). In principle the method involves 4 steps:-

1) Water extractable residues in the soil are determined.
2) The toxicity of the residues in the soil are assessed.
3) The dose response curve of the test species to the herbicide of interest is obtained in water culture.
4) The toxicity observed in (2) is compared with that predicted from (1) and (3).

Pestemer et al. (1980, 1983) have found excellent agreement between observed and predicted toxicity for atrazine, carbetamide, chloridazone, propyzamide, simazine, lenacil, monolinuron, linuron, propachlor and methabenzthiazuron in a wide range of vegetable crops. At WRO we have carried out experiments with atrazine, linuron, chlortoluron, metribuzin and propyzamide

using barley and rape as test plants on 5 occasions under different climatic conditions. Observed activity in the field was never greater than that predicted.

These results provide further evidence that any bound residues of herbicides that may exist are unlikely to be phytotoxic.

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FORMATION AND FATE OF BOUND RESIDUES OF BENZENE-^{14}C AND ITS CHLORINATED DERIVATIVES IN SOIL AND PLANTS

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Abstract

Outdoor experiments with hexachlorobenzene-^{14}C, pentachlorobenzene-^{14}C, 1,2,4-trichlorobenzene-^{14}C, and benzene-^{14}C in soil-crop-systems indicate that the formation rate of bound residues in soil and plants, expressed as bound residues in percent of total residue in the sample, decreases with increasing number of chlorine in the molecule, which is parallel to increasing resistance to chemical and biological degradation. The time-course of formation and fate of bound residues in soil and plants is characterized by a very slow decrease of residue levels in soil with time, indicating that biodegradation of bound residues hardly exceeds their re-formation from the parent compound during one vegetation period, and by a steep decrease of residue levels in plants, which, however, might be due rather to the "dilution" of residues by plant growth which offsets the small uptake from soil in later growth stages, than to biodegradation. The portion of bound residues as compared to the total residue increases with time, indicating that bound residues are more persistent than the parent compounds and their soluble metabolites; an exception for this observation is benzene. Cress plants, in general, contain less bound residues than barley plants, both in terms of concentration and in terms of percentage of total residue. Again, benzene is an exception; fast biological conversion in soil and plants to metabolites with other properties may be one reason for this exceptional behaviour. In deeper soil layers, soil-bound residues occur also. For the chemical group tested here, the ratio between bound and extractable residues does not differ to a larger extent between the soil layers.

Introduction

The dependence of the tendency of a pesticide to form bound residues in soil and plants upon chemical structure characteristics of the pesticide molecule has been reviewed recently (1). It is generally adopted today that this tendency is positively related to the susceptibility of the molecule to biological degradation and conversion, and/or to its chemical reactivity in general. However, information on this topic has been obtained mostly by comparison of experiments differing in experimental conditions, like soil type, pesticide concentration in soil, and climatic conditions.

The time-course of formation of bound residues in soil has been studied in the laboratory (2); information obtained under outdoor conditions is limited. Likewise, information is limited on the time-course of formation of bound residues in plants; this topic is very complex since levels of bound residues in the plant mass are governed not only by the interactions between formation and degradation rates of bound residues in plants but also by the uptake kinetics of the parent compound from the soil.

Although data are available on the dependence of the occurrence of soil-bound pesticide residues upon soil depth, they also have not been obtained under comparable environmental conditions.

The study presented here was carried out to investigate the influence of chemical structure characteristics on the formation rate of bound residues in soil and plants, the time-course of formation in soil and plants, and the occurrence of bound residues in deeper soil layers for a group of model compounds under identical experimental outdoor conditions. Since aromatic compounds probably constitute the bulk of pesticide-derived chemicals in agricultural soils, knowledge of the influence of substituents of the aromatic ring on the formation of bound residues is an essential precondition to assess total soil burden of bound chemicals after pesticide application. The fact that aromatic rings also are major constituents of natural humic substances gives further emphasis to the importance of aromatic compounds in

the chemical composition of soil. Therefore, a series of aromatic compounds comprising a wide range in chemical reactivity and biological persistence, including benzene, 1,2,4-trichlorobenzene, pentachlorobenzene and hexachlorobenzene, was selected for this study. Besides for its suitability as a model substance, hexachlorobenzene is interesting with respect to its use as a seed dressing (3). Pentachlorobenzene has been shown to be a metabolite of the insecticide lindane in soil and plants (4) and of the fungicide pentachloronitrobenzene in soil (5) and plants (6). 1,2,4-Trichlorobenzene has also been identified as a conversion product of lindane in plants (4), and benzene has been reported to be a conversion product of lindane in anaerobic soil (7).

Materials and Methods

The experiments were conducted under field-like outdoor conditions, as described earlier (8). The plants were grown in boxes 60 x 60 x 60 cm, constructed from water-resistant plywood. The base of the boxes contained holes to permit the drainage of excess water which was collected in a metal splash tray. The boxes were wrapped in aluminium foil on the outside to prevent temperature increases due to sunlight. The bottom 25 mm of the boxes was packed with stone chips about 25 mm in diameter, and the stones were covered with a 25-mm layer of sand. The boxes were filled with the same soil to 1 cm from the top. The soil was allowed to settle for 1 month before planting. The box was sunk into a large pit so that the upper surface of the soil was at the same level as the surrounding ground. Hexachlorobenzene-^{14}C, pentachlorobenzene-^{14}C, 1,2,4-trichlorobenzene-^{14}C and benzene-^{14}C were mixed with the corresponding inactive compounds and incorporated in the soil, resulting in a soil concentration of about 2 ppm to a 10-cm depth. In one half of each box, barley grains were sown, and cress in the other part of the box. This outdoor experimental set-up has been shown to yield residue data which are within the range of field value variations (8).

At appropriate time intervals, samples of both plant species as well as soil samples of the corresponding root zones were taken and analyzed for radioactivity. After the last harvest, soil samples were taken at definite depths (0 - 5 cm, 5 - 10 cm, 10 - 20 cm, 20 - 30 cm, 30 - 40 cm), in order to assess the influence of soil depth on the level of soil-bound residues.

For the determination of bound residues, the plants were homogenized in methanol with an Ultra-Turrax, then extracted with methanol in a Soxhlet for 48 hours. Fresh soils were also extracted in a Soxhlet for 48 hours. All unextractable residue data presented in this paper refer to this extraction method, with the exception of some soil samples containing benzene-^{14}C residues, which were extracted with cold methanol; this is marked in the respective tables. After the extraction, the radioactivity in the extracts was determined by liquid scintillation counting; unextractable radioactivity left in plant and soil samples was determined by combustion of the samples, followed by liquid scintillation counting of $^{14}CO_2$ trapped in a suitable scintillation cocktail.

Influence of Chemical Structure on the Formation of Bound Residues in Soil and Plants

Figure 1 gives comparative data for unextractable residues in soil and plants, one vegetation period after the application of hexachlorobenzene-^{14}C, pentachlorobenzene-^{14}C, 1,2,4-trichlorobenzene-^{14}C, and benzene-^{14}C into the soil, in µg equivalent to the parent compound per g dry soil or plant material.

It has been shown that the formation of bound residues in soil and plants is negatively correlated to the number of chlorine atoms in the molecule (1). Contrary to this observation, this table which presents absolute bound residue levels in terms of ppm, demonstrates a weak decrease of bound residues in soil with decreasing chlorine content of the chemicals and a strong decrease in plants. However, it should be kept in mind that these four substances are more or less volatile and that volatilization

is a process which competes very effectively both with the process of binding in soil and with the uptake by plants; its rate may exceed both soil binding rates and rates of plant uptake followed by binding in plants. Therefore, absolute concentration figures in this case are not suitable for comparative assessment of binding capacity of chemicals to soils or plants. The ratio between bound and soluble residues is a much better means to compare the tendency of chemicals to form unextractable residues in soils or plants.

Figure 2 gives the same data as Table I, not in terms of concentration figures but as bound residues in percent of total residues in the respective sample. This table shows a strong negative correlation between the degree of chlorine substitution of benzene and binding in soils and plants, with the exception of the value for benzene in barley, which does not fit this scheme. However, we should keep in mind that it is questionable whether benzene-derived ^{14}C taken up by plants is still the unchanged parent compound. It is much more probable that not benzene itself is taken up by plants, but some degradation products of benzene in soil, which of cause may have quite other plant-binding properties than benzene. The fact that cress plants do not show this irregularity is in accordance with the fact that the lipid content of this plant is up to 2 % of dry mass. This means that these plants could have, besides the normal uptake route via the conduction channels, another possibility to take up unchanged lipophilic chemicals from soils and to accumulate them in oil cells, where they are not accessible to metabolism. It is interesting that the formation of plant-bound residues by chlorinated benzenes, a metabolic process, is much lower in cress than in barley, the differences between both plant species being highest for hexachlorobenzene, a compound with very low water solubility, and lowest for 1,2,4-trichlorobenzene with a higher water solubility; only for benzene, the formation of bound residues in cress is higher than in barley.

Influence of Time on the Level of Bound Residues in Soil and Plants

In the following section, the time-course of formation and fate of bound residues in soil and plants is discussed for each chemical separately.

Hexachlorobenzene

Figure 3 shows soil- and plant-bound residues of hexachlorobenzene in µg/g dry weight at different time intervals. Since hexachlorobenzene is known as a very persistent compound, the levels of unextractable residues in soil, resulting from metabolic processes, are as low as expected. There is no distinct time dependence for soil-bound residues, indicating some kind of equilibrium between formation and degradation of bound residues. In barley plants, there is also a very slow decrease of residues although the decrease of total residues including extractable radioactivity is very steep (9). In cress plants, the unextractable residues decrease by one order of magnitude within the growing period of 79 days. In Figure 4, the same data are expressed as relative figures, i.e. as bound residues in terms of percent of total residue in each sample. The increase of these data with time is less pronounced for soil and cress plants, but higher than one order of magnitude for barley. It seems that barley, similar to wheat (10), is able to metabolize this persistent compound to a considerable extent by enzymatic activity.

Pentachlorobenzene

The levels of bound residues of pentachlorobenzene in soil (Figure 5) are similar to those of hexachlorobenzene. Barley and cress plants exhibit a pronounced decrease of bound residue levels during the vegetation period, which parallels the decrease of total residue levels including extractable radioactivity (9). Both plants probably take up the chemicals preferably in their first growing stage; during further growth, the residues decrease by "dilution" as a consequence of an increase in plant mass.

"Relative" bound residues, expressed as percent of total residue of each sample, show a positive correlation with time for soil and both plant species (Figure 6).

1,2,4-Trichlorobenzene

Bound residues of 1,2,4-trichlorobenzene, expressed as ug equivalent to the parent compound per g dry weight (Figure 7), in soil apparently do not change with time; in both plant species bound residues decrease with time. The relative data expressed as bound residues in percent of total residue present in each sample increase with time (Figure 8).

Benzene

The levels of bound residues derived from benzene in soil and plants (Figure 9) are very low already at the first sampling (12 days) and decrease further during the vegetation period, in spite of the fact that benzene is a readily biodegradable compound. In fact, both volatilization and total degradation to carbon dioxide are so fast that only low residues, both bound and soluble, are left. In cress, the levels of bound residues are higher than in barley; this is in contrast to the three chlorinated benzenes discussed above. The unextractable residue portion is between 55 and 68 % of total residues in soil and 27 and 100 % in plants (Figure 10) and does not show any clear time dependence, neither for soil nor for plants. This means that both kinds of residues are formed and degraded at a comparable rate and bound residues derived from benzene are hardly more persistent than extractable residues. In cress, the portion of unextractable residues as compared to total residues is higher than in barley; this is in contrast to the three chlorinated benzenes discussed above.

Influence of Soil Depth on the Level of Bound Residues in Soil

After the harvest of all plants of these experiments, soil samples were taken at definite depths (0 - 5 cm, 5 - 10 cm, 10 - 20 cm,

20 - 30 cm, 30 - 40 cm), in order to assess the influence of soil depth on the level of soil-bound residues of benzene and its chlorinated derivatives. Table I shows the levels, in % of initially applied radioactivity, for the four test compounds and the six different depths. The table shows the presence of bound residues to a depth of 40 cm for hexachlorobenzene, 1,2,4-trichlorobenzene and benzene, and the difference in concentration between top soil and lowest soil layer is not very great. If the distribution rate of bound residues in soil is expressed in terms of residues below 10 cm as a percentage of total residue in soil, then we get 51.0 % for hexachlorobenzene, 32.7 % for pentachlorobenzene, 71.2 % for 1,2,4-trichlorobenzene, and 63.5 % for benzene.

The formation of soil-bound residues in soil layers below the treated 0 - 10 cm top soil depends upon the migration rate of the parent compound, which in turn depends on its water solubility, and upon the formation rate of bound residues which is influenced, as discussed above, by its biodegradability. Both parameters - water solubility and biodegradability - are highest for benzene and lowest for hexachlorobenzene. Consequently, benzene should give higher bound residues in deeper soil layers than the other three test substances. In fact, the experiments described here show that this is not true; the percentage of bound residues in deeper soil layers as compared to the top soil is highest for 1,2,4-trichlorobenzene and lowest for pentachlorobenzene. It should be considered that in top soil volatilization and total biodegradation to carbon dioxide may overcome leaching processes; in deeper soil layers, total biodegradation is also a competitive process so that a compound with lower water solubility and higher biological persistence (1,2,4-trichlorobenzene) may form higher levels of soil-bound residues in deeper soil layers than a non-persistent compound with a higher water solubility (benzene). The formation of soluble metabolites in soil, which may have water solubilities and biological persistences quite different from those of the parent compound, further complicates the prediction of leaching and binding processes.

The ratio between soluble and bound residues, expressed as bound residues in percent of total radioactivity in each sample (Table II), shows no marked differences between various soil depths.

Further Investigations in Progress

These experiments which give informations on binding kinetics and dependence upon chemical substance structure, do not give much indications on kind and mechanisms of binding, except for the finding that their persistence in soil indicates that they do not consist merely of assimilated natural products, at least for the three chlorinated substances. For a thorough investigation of these questions, these four model chemicals turned out to be not suitable since bound residue levels are very low, either due to high persistence or to high volatility of the parent compounds.

Therefore, we have started further experiments with two aromatic compounds (one phenol and one aniline) and, for comparison, one non-aromatic compound to study the relation between amounts bound in the inorganic and in the organic soil fractions (11). For this purpose, the treated soils are fractionated according to Helling and Krivonak (12). As a reference compound, glucose-^{14}C is subjected to the same procedure, in order to assess the portion of bound residues which consists of assimilated natural biological material. First results for the phenol show that the amounts bound in the mineral and organic fractions are comparable; however, since the mass of inorganic fractions constitutes more than the tenfold of that of the organics, the concentration of bound residues in the organic fractions is higher than that in the inorganic fractions by more than one order of magnitude; thus, the organic soil fraction accumulates organic xenobiotics from soil as a total in a manner comparable to the accumulation of xenobiotics from water by water organisms.

Since mineral and organic fractions represent quite different forms of binding, it is planned to study biodegradability, photodegradability and plant availability for each of these fractions separately.

Conclusion

We may conclude that the strong relationship between chemical structure and binding processes in soil and plants, as documented by these experiments, gives evidence for the participation of chemical reactions in the binding processes, which we hope to elucidate further by our experiments in progress.

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Table 1. Distribution of Bound Residues in Different Soil Layers, 126 Days after Treatment of Soil with Benzene-^{14}C and Chlorinated Benzenes-^{14}C (in % of applied radioactivity)

Soil depth (cm)	Hexachlorobenzene	Pentachlorobenzene	1,2,4-Trichlorobenzene	Benzen
0 - 5	0.32	0.43	0.23	0.16
5 - 10	0.16	0.23	0.17	0.11
10 - 20	0.32	0.32	0.38	0.20
20 - 30	0.09	n.d.	0.37	0.15
30 - 40	0.09	n.d.	0.24	0.12
Soil, total	0.98	0.98	1.39	0.74

n.d. = none detected

* Residues after cold extraction with methanol

Table 2. Bound Residues in Different Soil Layers, 126 Days after Treatment of Soil with Benzene-^{14}C and Chlorinated Benzenes-^{14}C, in Relation to Extractable Residues (bound residues in % of total radioactivity of each sample)

Soil depth (cm)	Hexachlorobenzene	Pentachlorobenzene	1,2,4-Trichlorobenzene	Benzene
0 - 5	0.8	2.0	23.0	100.0
5 - 10	0.9	2.1	19.3	100.0
10 - 20	1.2	4.3	28.5	100.0
20 - 30	1.1	n.d.	35.0	100.0
30 - 40	0,7	n.d.	38.0	100.0

n.d. = none detected

* Residues after cold extraction with methanol

FIG. 1

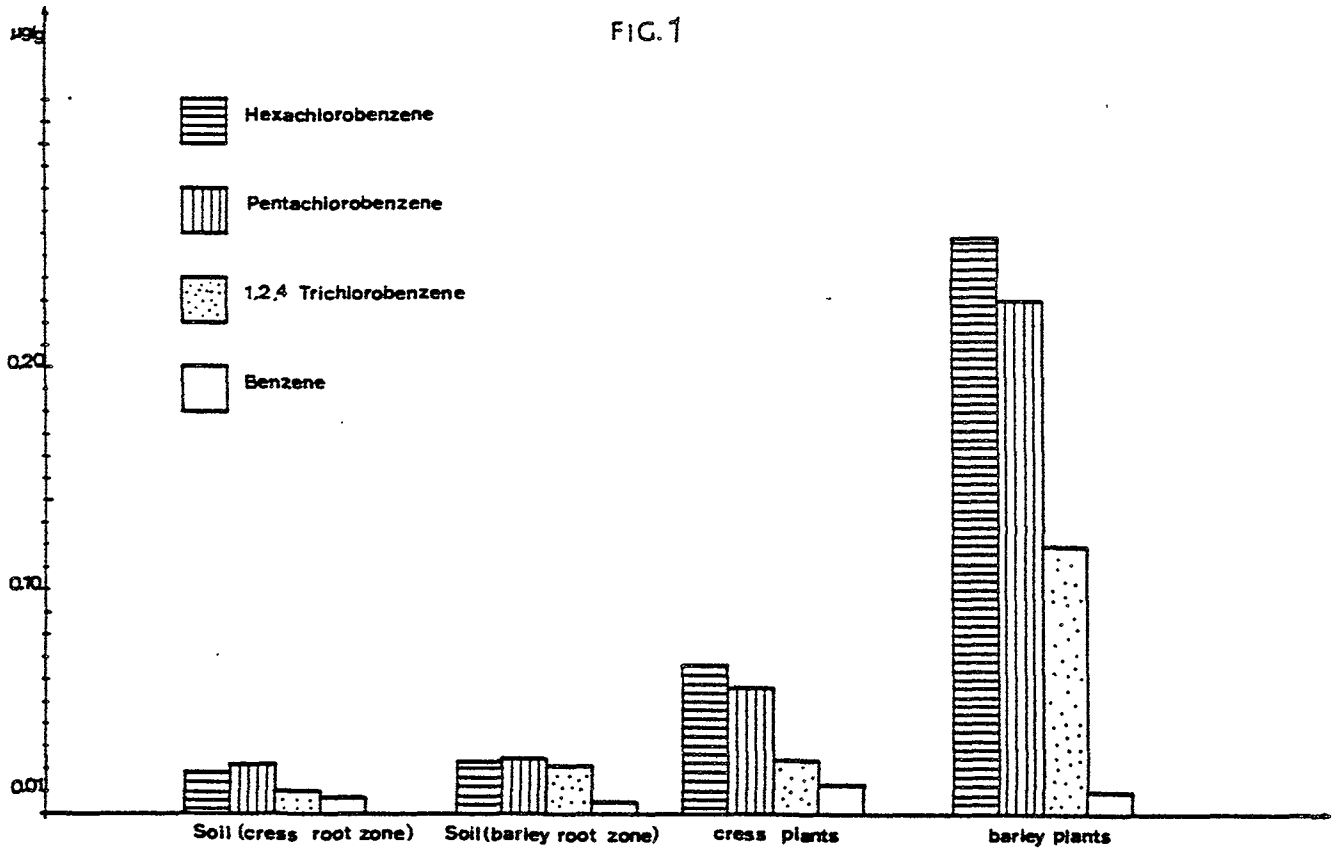

Fig. 1. Unextractable radioactive residues in soil and plants after treatment of soil with benzene-^{14}C and chlorinated benzenes-^{14}C (in $\mu g/g$ dry weight; for cress and cress soil, 77 - 79 days after soil treatment; for barley and barley soil, 124 - 125 days after soil treatment)

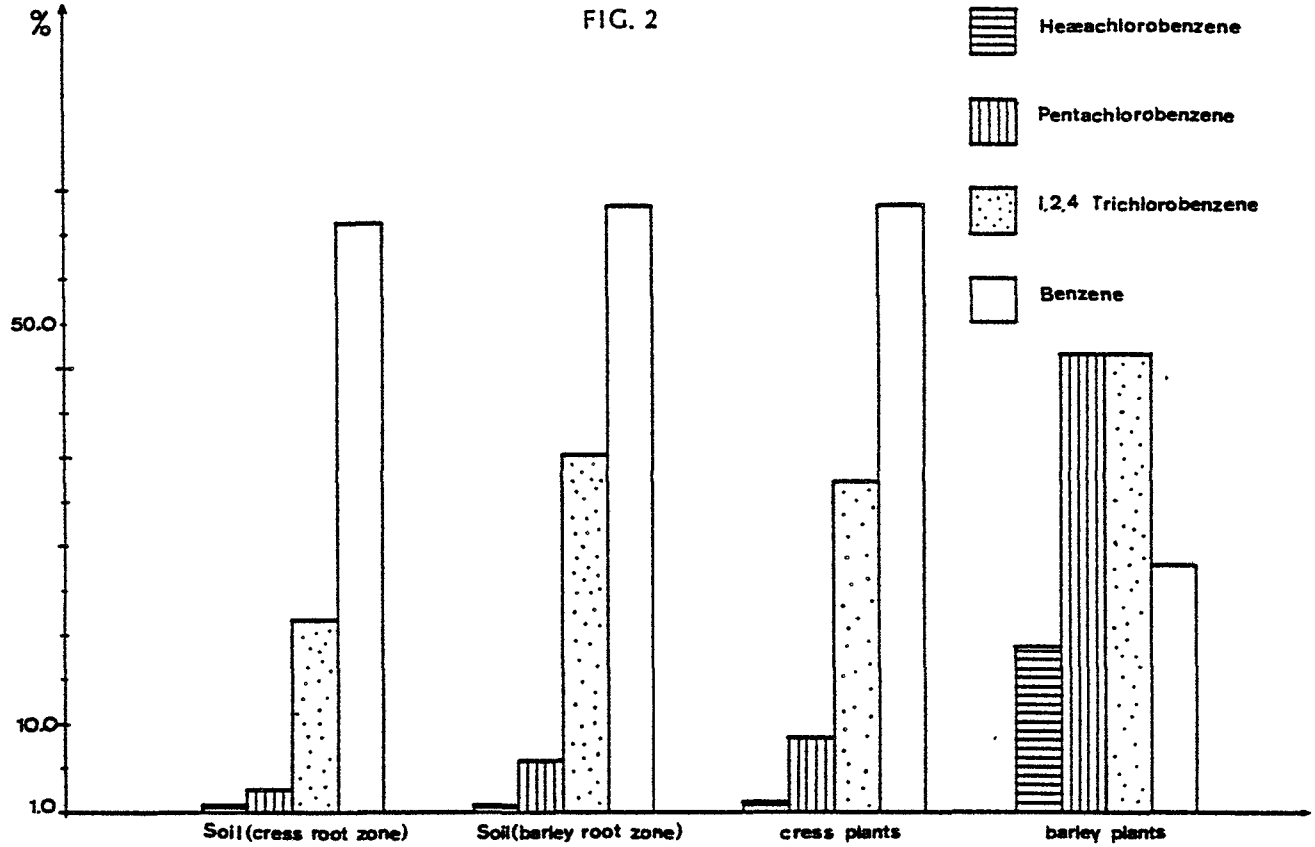

Fig. 2. Unextractable radioactive residues in soil and plants after treatment of soil with benzene-^{14}C and chlorinated benzenes-^{14}C, in relation to extractable residues (bound residues in % of total radioactivity of each sample; for cress and cress soil, 77 - 79 days after soil treatment; for barley and barley soil, 124 - 125 days after soil treatment)

FIG. 3

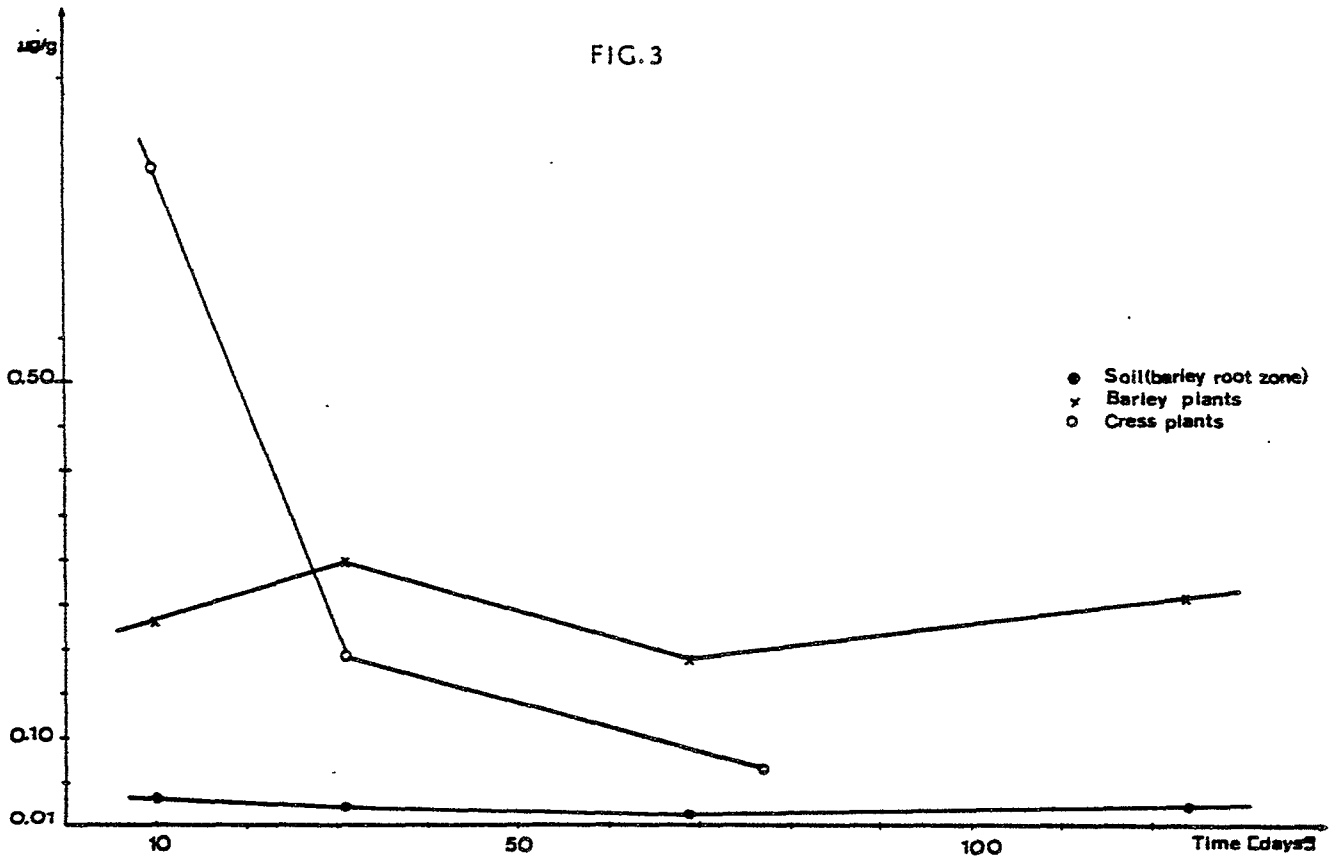

Fig. 3. Time-course of formation of bound residues in soil and plants after treatment of soil with hexachlorobenzene-^{14}C (in µg/g dry weight)

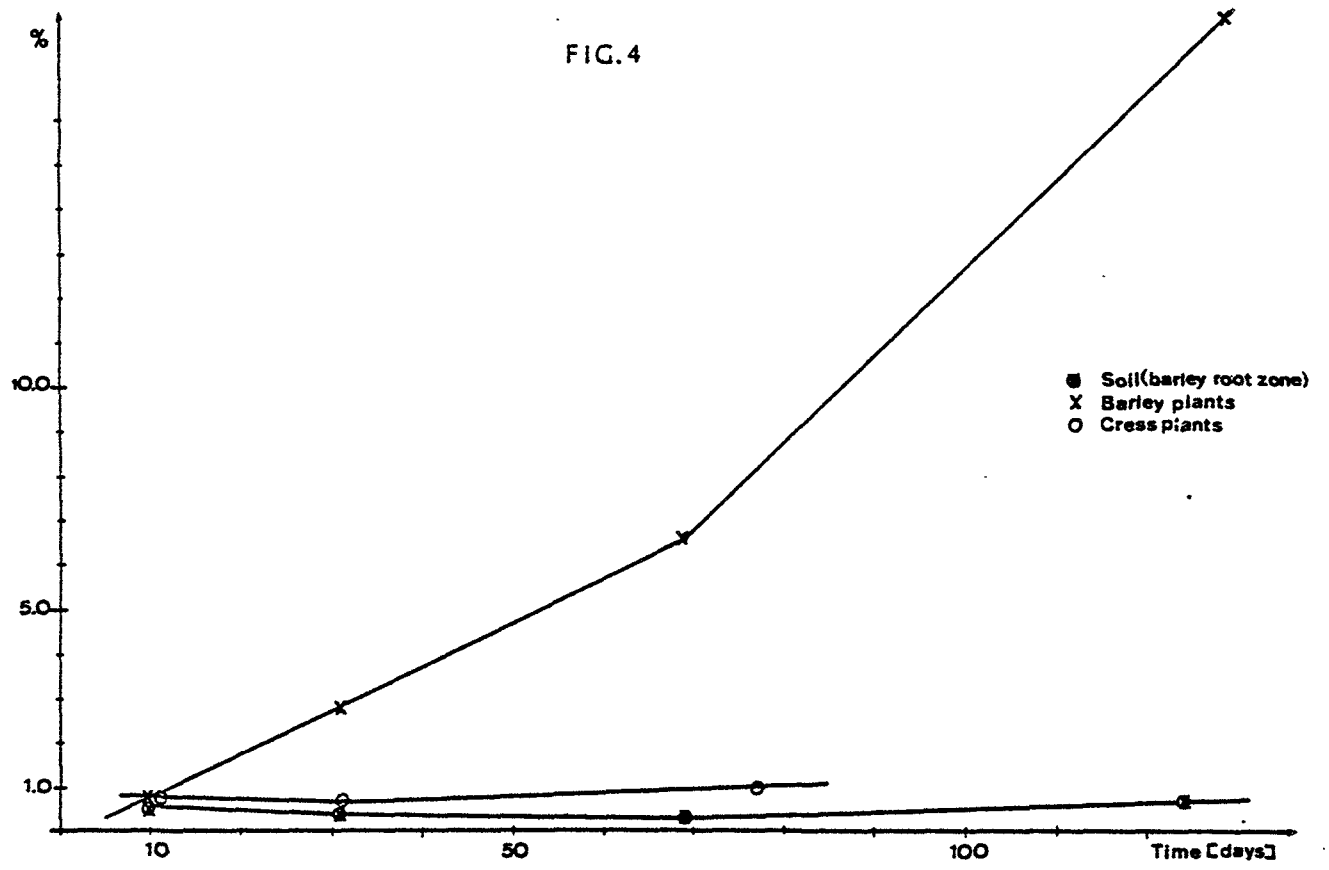

Fig. 4. Time-course of formation of bound residues in soil and plants after treatment of soil with hexachlorobenzene-^{14}C, in relation to extractable residues (bound residues in % of total radioactivity of each sample)

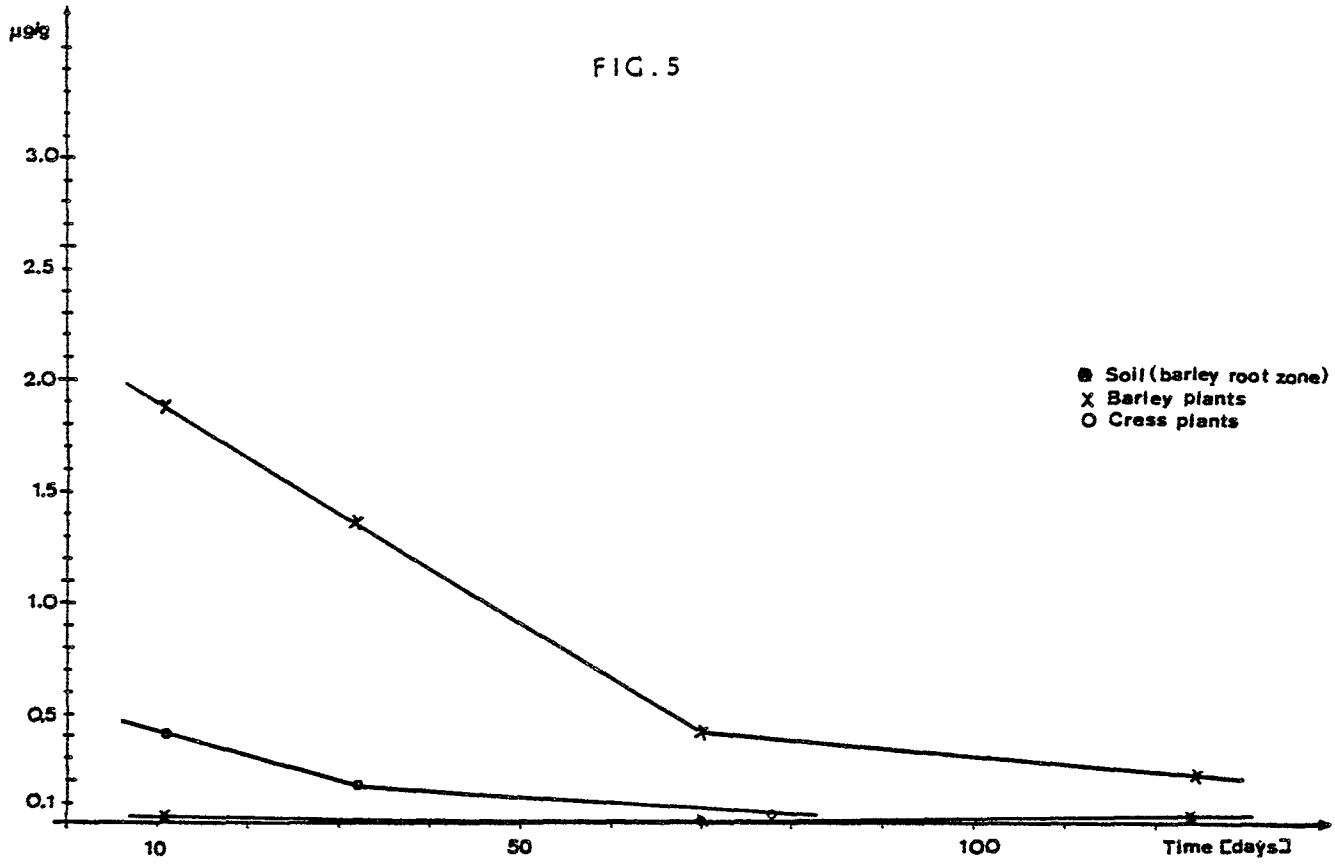

Fig. 5. Time-course of formation of bound residues in soil and plants after treatment of soil with pentachlorobenzene-^{14}C (in µg/g dry weight)

FIG. 6

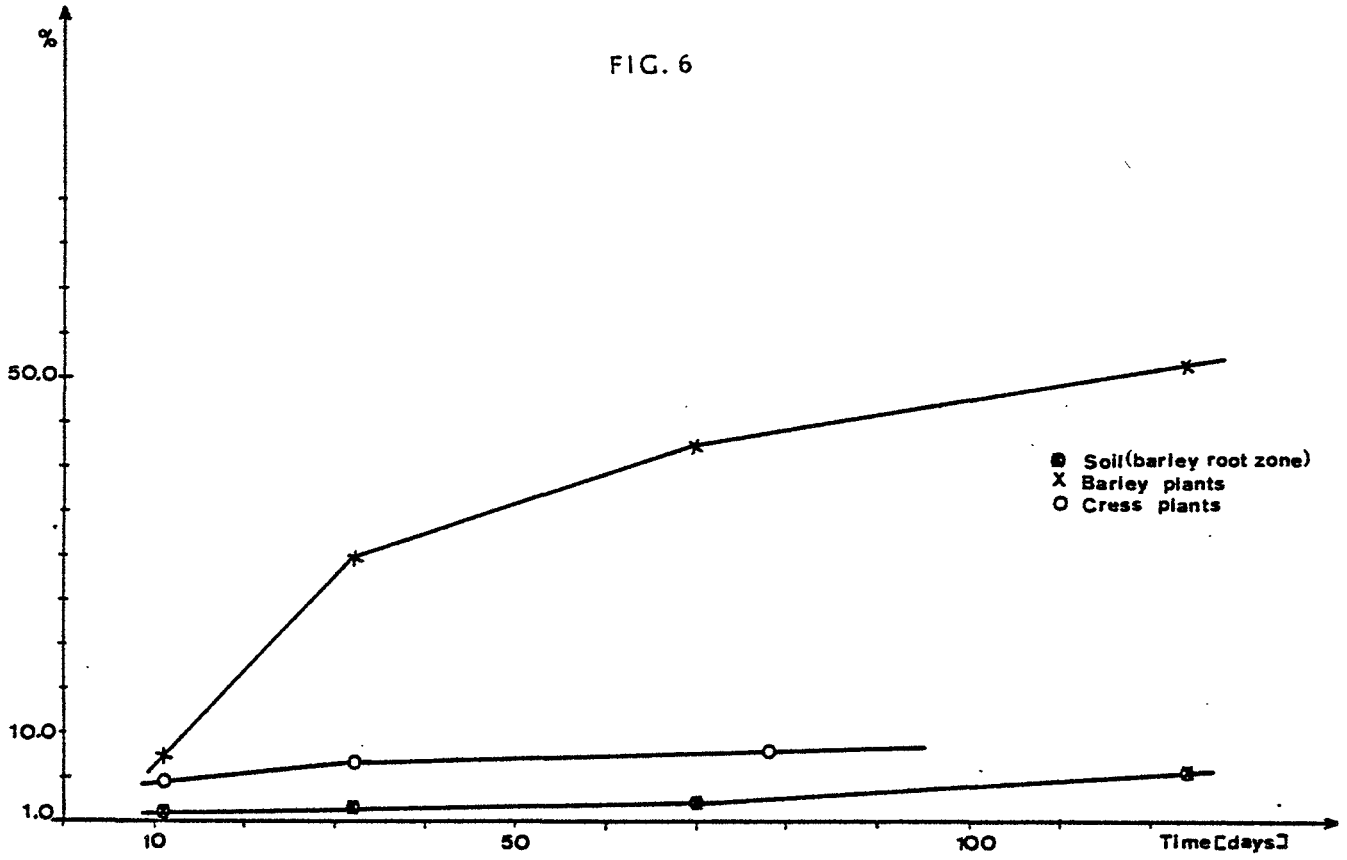

Fig. 6. Time-course of formation of bound residues in soil and plants after treatment of soil with pentachlorobenzene-^{14}C, in relation to extractable residues (bound residues in % of total radioactivity of each sample)

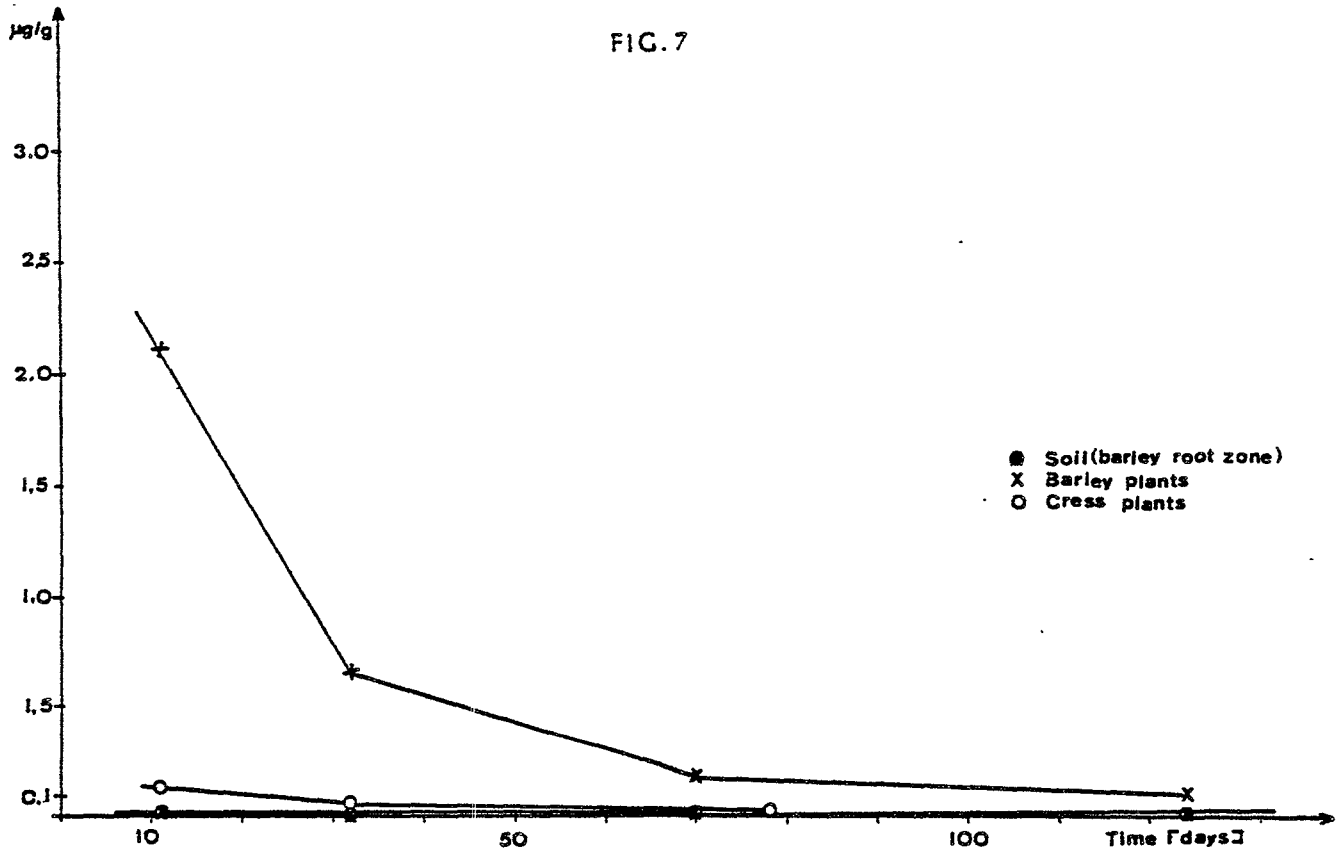

Fig. 7. Time-course of formation of bound residues in soil and plants after treatment of soil with 1,2,4-trichlorobenzene-^{14}C (in µg/g dry weight)

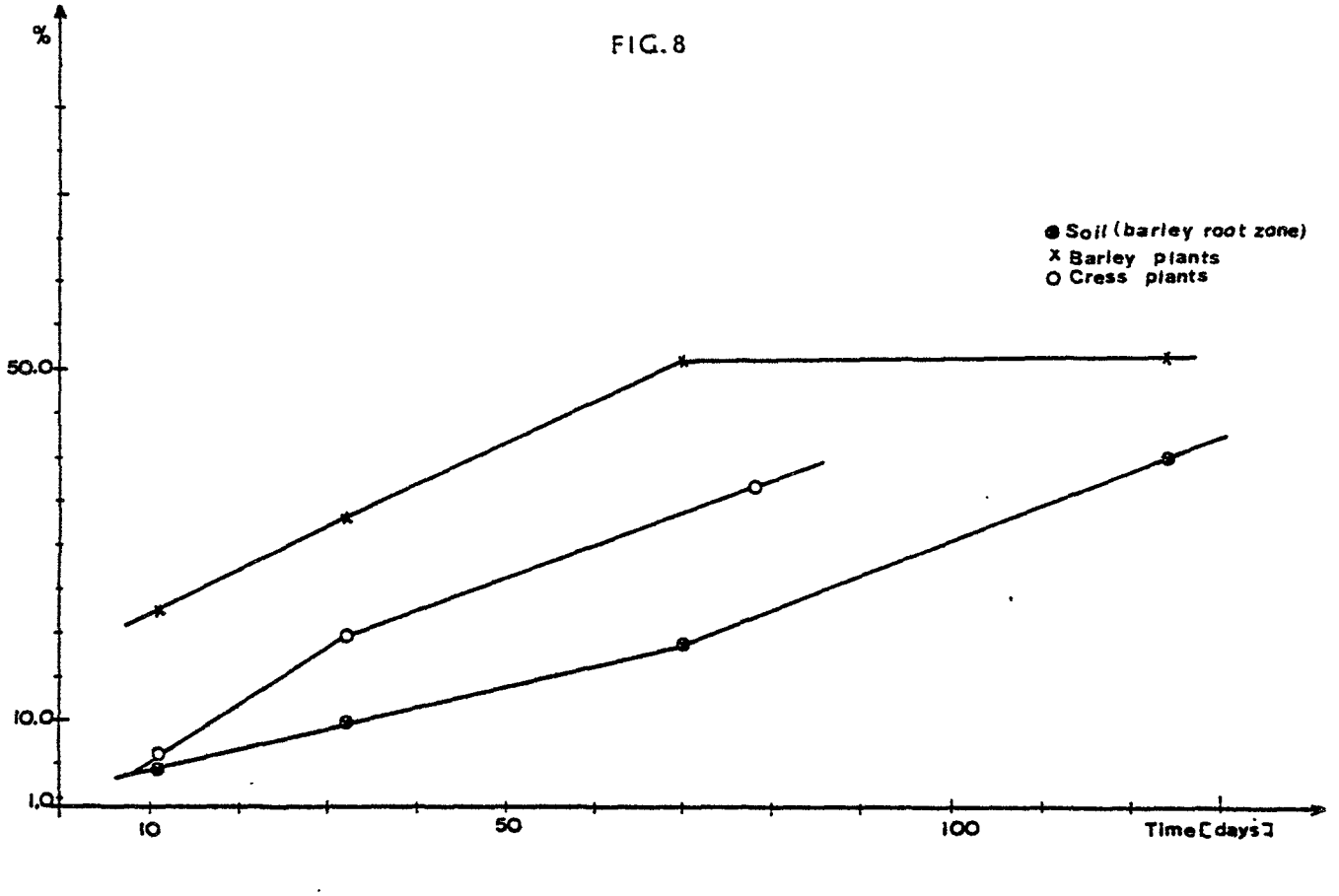

Fig. 8. Time-course of formation of bound residues in soil and plants after treatment of soil with 1,2,4-trichlorobenzene-^{14}C, in relation to extractable residues (bound residues in % of total radioactivity of each sample)

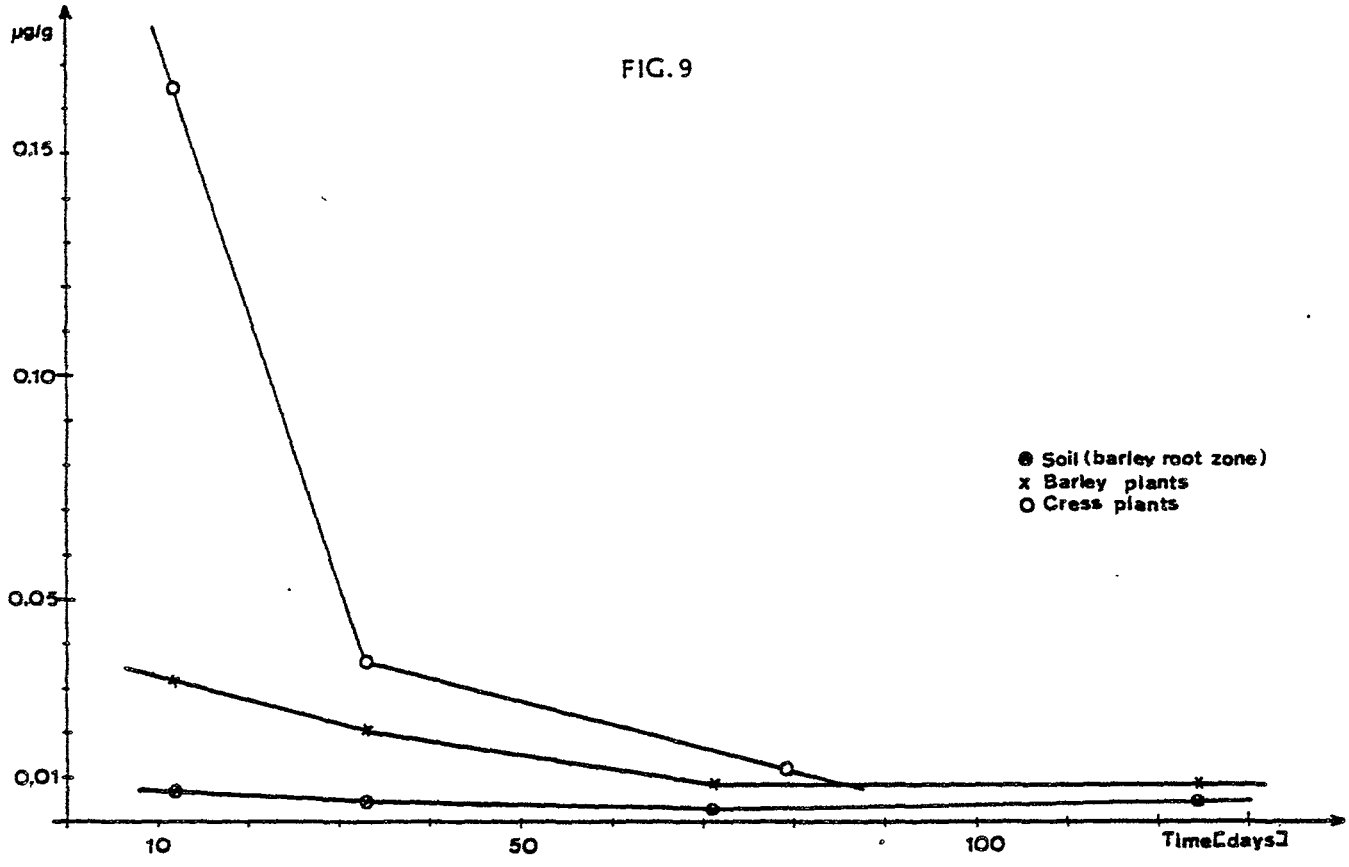

FIG. 9

Fig. 9. Time-course of formation of bound residues in soil and plants after treatment of soil with benzene-^{14}C (in µg/g dry weight)

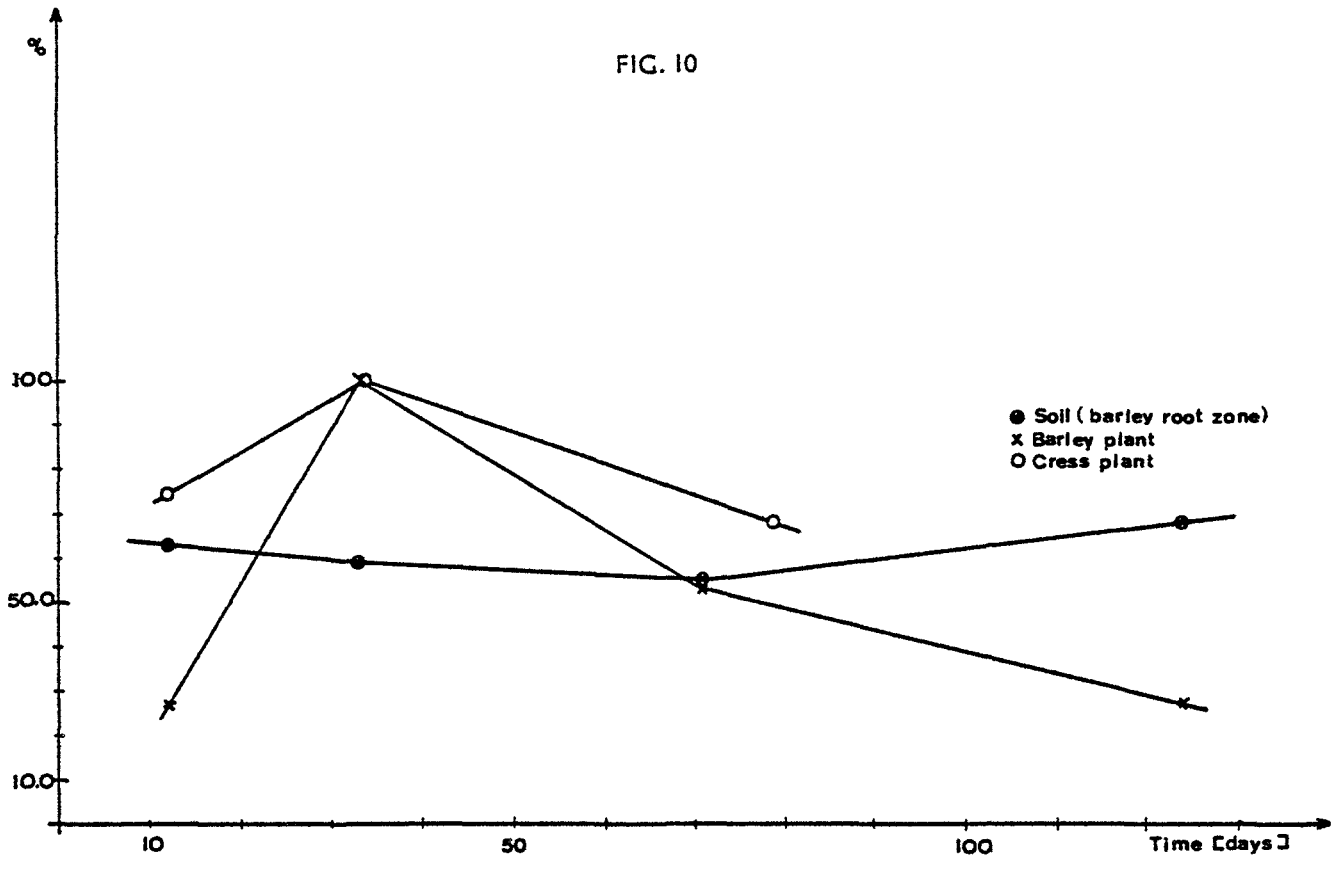

Fig. 10. Time course of formation of bound residues in soil and plants after treatment of soil with benzene-^{14}C, in relation to extractable residues (bound residues in % of total radioactivity of each sample)

FATE OF DIELDRIN IN RADISHES

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Abstract

^{14}C-dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene) was applied in a commercial dieldrin formulation to the roots and surrounding soil of early mature radishes, maintained in environmental growth chambers, at rates of 1.1 and 11.1 kg/ha. Edible portions of the radishes were sampled 1, 7, 14 and 21 days post application. ^{14}C residues (as dieldrin equivalents) present in the 1.1 kg/ha treatment ranged from 0.4-2.1 ppm, and in the 11.1 kg/ha treatment from 4.0-8.5 ppm. Extractable ^{14}C was primarily dieldrin (77-94%) and photodieldrin (2.7-10.4%). The concentration of unextractable ^{14}C increased with time for both treatments (0.11-0.96 ppm). Much of the bound ^{14}C was released by acid hydrolysis of the solvent extracted tissue. Radiocarbon associated with lignin remained relatively constant for the 1, 7 and 14 day post application periods (approximately 0.02 ppm), but a large increase occured between 14 and 21 days (approximately 0.27 ppm).

Dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene) is a chlorinated hydrocarbon insecticide which was registered for use in the United States as an insecticide. It is considered to be relatively persistent in the environment and is not metabolized extensively by plants or animal enzyme systems.

Major mechanisms for plants to contain dieldrin residues are root absorbed translocation from soil and absorption through the leaves (either from direct application or volatilization from the soil). Translocation of root absorbed dieldrin occurs in red mangrove seedlings (Walsh et al., 1974), corn (Beestman et al., 1969) and grass plants (Voerman and Besemer, 1975). Aerial contamination by absorption through the leaves is more pronounced when field grown corn is compared to greenhouse grown corn which had been protected from aerial contamination (Barrows et al., 1969). Volatilization of dieldrin from soil over a growing season and absorption through the leaves of corn, combined with root absorption, results in residues up to 1.33 ppm (Taylor et al., 1976). The availability of dieldrin for absorption by plants is increased by increasing the water content of the soil (Saha et al., 1971) and decreasing the soil organic content (Saha et al., 1971; Wheeler et al., 1967b; Harris and Sans, 1972 and Nash et al., 1970). Wheeler et al. (1967b) observed 2 to 6 times higher levels of dieldrin in forage plants grown in sand than plants grown in soil. Quantitative extraction of absorbed dieldrin from forage plants was achieved using chloroform:methanol (1:1) (Wheeler et al., 1967a). Photodieldrin is the major metabolite detected (5-10%) in cabbage, spinach and carrots (Weisgerber et al., 1970).

Nonextractable (or bound) pesticide residues are defined as chemical species originating from pesticide usage that cannot be extracted by methods commonly used in residue analyses and metabolism studies. This definition was formulated by the First FAO/IAEA Research Coordination Meeting on isotopic tracer-aided studies of bound pesticide residues in soil, plants and food. Generally, chlorinated hydrocarbon insecticides are not often reported to form bound residues in plant substrates. In one instance, however, Nash et al.(1970) reported for

soybeans grown in soil containing ^{14}C dieldrin, that as much as 40% of the ^{14}C in the beans and 9% in the hay was unextractable using chloroform:methanol as the extracting solvent. Wheeler and Thompson (1981) have noted the presence of bound ^{14}C when radiolabeled dieldrin was applied in a commercial formulation to the roots and surrounding soil of early mature radishes. Twenty to thirty percent of the ^{14}C could not be extracted by blending with either acetone, acetonitrile or methanol. This phenomenon noted with dieldrin resulted in efforts to determine: 1) uptake and metabolism of dieldrin at various application rates, 2) extractability of the absorbed dieldrin, 3) levels and percentages bound and 4) some characteristics of the bound material.

MATERIALS AND METHODS

Chemicals. Uniformly ring-labeled ^{14}C dieldrin (Amersham Corporation, 85 mCi/mmol) was used to fortify a Shell emulsifiable concentrate (EC) containing 18% technical dieldrin. Three application rates were used: 1.1, 11.1 and 111 kg/hectare. The 111 kg/hectare treatment solution was prepared by mixing 16.39 g (3.06 g technical dieldrin) of the formulation with 6.72 mg ^{14}C dieldrin in acetone (1.5 mCi) and diluting to 300 ml with H_2O for a final specific activity of 0.19 mCi/mmol. Dilutions of this solution were made to prepare the 1.1 and 11.1 kg/hectare stock solutions. All solvents used were pesticide grade. Dieldrin (99.7%) and photodieldrin (98 %) analytical standards were obtained from the Environmental Protection Agency (Research Triangle, North Carolina). Trans-aldrindiol was synthesized by the H_2SO_4 hydrolysis of dieldrin (Korte and Arent, 1965) with the addition of 1,4 dioxane to enhance the yield (Mckinney et al., 1971). Pepsin powder from porcine stomach mucosa with an activity of 1200-2000 units per mg protein was used as received (Sigma).

Chromatographic and Radioassay Procedures. Thin layer chromatography (TLC), carried out using silica gel 60 F-254 chromatoplates (0.25 X 20 cm, E. Merck), employed two solvent systems: methylene chloride and n-heptane:acetone (80:20). Gas chromatography (GC) was done on a Hewlett Packard Model 5840 fitted with a ^{63}Ni electron capture detector (ECD) and a flame ionization detector (FID). Argon/methane (5:95) was the carrier gas for ECD with a flow rate of 60 ml/min. Nitrogen was the carrier gas for FID and had a flow rate of 30 ml/min. The air and hydrogen flows for FID were 300 ml/min and 30 ml/min, respectively. The injector and detector temperatures were 225°C and 350°C, respectively. Glass columns (1.8 m X 2.0 mm id) were packed with 3% QF-1, 1.5% OV-17 + 1.95% QF-1 (on 100/120 mesh Gas Chrom Q) and 2% SP 2330 (on 100/120 Chromosorb) and were operated between 180°C to 210°C. Electron impact GC mass spectrometry (MS) was performed on a Finnigan 4021 GC/MS, fitted with a 1.8 m X 2.0 mm id glass column packed with 3% QF-1 (on 100/120 mesh Gas Chrom Q) and operated at isothermal and programmed temperatures between 155°C-220°C. GC/MS was done to authenticate the reference compounds.

Radish tissues remaining after extraction and the hydrolytic treatments were dried at 105°C for 24 hours. Radiocarbon determinations in all tissues were made by combustion on a Packard Tri-Carb Sample Oxidizer B 306 . All liquid scintillation counting (LSC) was done on a Searle Analytic 92 liquid scintillation counter utilizing Aquasol II plus 10% water. The cpm were converted to dpm using quench curves.

Treatment and Analysis of Plants. Red Globe radish seeds were germinated in flats containing soil and then transplanted to 14.6 cm pots (4/pot) containing sand at 1.5-2 weeks of age. The flats and pots

were maintained in an environmental growth chamber (Scherer-Gillet Model CEL 512-37), with 10 hour light periods and 14 hours of dark. Light and dark temperatures were 27°C and 16°C, respectively; light and dark relative humidities were 80% and 60%, respectively. The radishes were treated at 5-6 weeks after germination by pipeting 5 ml of the ^{14}C fortified formulated dieldrin onto the roots and surrounding soil of each radish (total of 20 ml per pot) at rates of 1.1, 11.1 and 111 kg/hectare. An untreated control was maintained in the same growth chamber. The sand was covered with paraffin shavings immediately after dieldrin application to reduce volatilization of the insecticide.

The radishes were harvested 1, 7, 14 and 21 days post application by pulling them from the sand, rinsing them with water to remove adhering sand and removing the tops. They were immediately weighed and then chopped with a hand operated food chopper to particles of 0.5-1.0 mm in size prior to analysis. The radish weights from the various treatments were analyzed statistically by a one way analysis of variance. Dunnett's procedure was used to compare means of treatment groups to controls and Tukey's procedure was used to compare treatment means.

The chopped radish tissues were extracted in a Soxhlet extractor for 24 hours (an average of two cycles per hour) with chloroform:methanol (2:1). The solvent-extracted radish tissues were dried; the extracts were transferred to separatory funnels, 100 ml of distilled water was added and the funnels were shaken. After separation of the aqueous and organic phases, the aqueous phases were re-partitioned with 50 ml of chloroform, which was then combined with the first chloroform phase. Both the aqueous and organic phases were assayed for ^{14}C by LSC. The organic phases were then concentrated on a rotory evaporator for another ^{14}C determination and evaluation of

^{14}C components by TLC. Benzene:ethanol (2:1) was also used to extract portions of the chopped radish tissue as the initial step in the Association of Official Analytical Chemists (AOAC) indirect lignin analysis (1970).

Portions of the extracted dry tissue were subjected individually to the various hydrolytic treatments shown in Table I. After the AOAC indirect lignin analysis, only lignin and ash remained. The soluble fractions from the hydrolytic treatments were partitioned with methylene chloride. They were then neutralized with sodium hydroxide and again partitioned with methylene chloride, and finally they were made alkaline with sodium hydroxide and again partitioned with methylene chloride. The radiocarbon in the methylene chloride fractions and that remaining in the aqueous hydrolysate was determined.

Identification of Conversion Products. The organic phase concentrates were streaked on TLC plates and developed to 10 cm. Areas of ^{14}C were detected by placing the plates in contact with Kodak No-Screen X-Ray film for autoradiography. The separated zones were scraped, eluted using Aquasol II + 10% H_2O scintillation cocktail and counted to determine the percentage distribution of ^{14}C. Also, ^{14}C zones from TLC were eluted with acetone, methanol or methylene chloride for GC and GC-MS analysis. Chromatographic behavior of reference compounds was compared to the ^{14}C zones.

RESULTS AND DISCUSSION

Radish Physical Parameters. In an effort to detect effects of dieldrin on radish physiology, the weights of the roots were measured. The mean radish weight one day post application was 4.6 g (all statistically equivalent at the 0.01 confidence level) for all application rates and the untreated control. The mean weight of radishes

treated at the 1.1 and 11.1 kg/hectare rates, including the control, increased to 25.9 g (0.01 confidence level) by 21 days post application at a nearly linear rate. Radishes treated at the 11.1 kg/hectare rate averaged 5 grams less than the control and 1.1 kg/hectare treatments through the 7 and 14 day harvests but they had attained an equivalent mean weight by 21 days post application. The 111 kg/hectare radishes did not grow or increase in weight, remaining at 5 grams for all harvest periods. Due to the inhibition of growth and obvious visual signs of toxicity as a result of this treatment rate, the data for the 111 kg/hectare treated radishes will not be discussed.

Other physical parameters of the radishes which were measured were: percentage H_2O, extracted dry weight, volume to weight ratio and lignin content at the various harvest intervals. The percentage H_2O remained constant through treatment rates and harvest intervals, ranging from 92 to 95%. The percentage of tissue residue remaining after extraction also remained constant over treatment rates and harvest intervals, ranging between 3.0 and 4.0%. The volume to weight ratio of the radishes at the 1, 7 and 14 day harvest periods (4-6 weeks post germination) was approximately 1.00 and increased to 1.29 at 21 days. Amounts of lignin present (mg lignin/g wet weight) again were relatively constant for the 1, 7 and 14 day harvest intervals at approximately 8.3 mg/g, but had increased to 11.8 mg/g at 21 days post application. The 22% increase in the volume to weight ratio between 14 and 21 days corresponded to higher amounts of lignin and visual signs of plant senescence.

Total Uptake of Dieldrin. Concentrations of ^{14}C, calculated as dieldrin equivalents, detected in the radishes at the time of harvest for the 1.1 and 11.1 kg/hectare rates are presented in Table II.

Control radishes contained between 0.5 and 1.0 ppm which may have been caused by cross contamination through volatilization of dieldrin and subsequent absorption through the leaves. These levels were used to normalize the 1.1 and 11.1 kg/hectare treatment rate data. Levels at the 1.1 kg rate ranged from 0.4 to 2.1 ppm and at the 11.1 kg rate, from 4.0 to 8.5 ppm. Reductions in ppm as time increased post application were caused by radish growth. Even though the ppm levels present decreased or remained relatively constant over the 21 day interval, the absolute amount of dieldrin residues increased. The recovery of applied radiocarbon from the plants (edible portion of the roots) at the various harvest intervals as a percentage of applied dose increased for both the 1.1 and 11.1 kg/hectare rates (see Table III).

Extractable Radiocarbon. The total ppm extractable radiocarbon expressed as dieldrin equivalents, for the 1.1 and 11.1 kg/hectare rates at 1, 7, 14 and 21 days are shown in Table II. At the the 1.1 kg/hectare rate 60-90% of the ^{14}C was extractable and at the 11.1 kg/hectare rate, 76-97% was extractable.

The percentage distribution of dieldrin and conversion products in the extractable fraction (organic phase) are shown in Table IV (there was no detectable ^{14}C in the aqueous phase). Dieldrin ranged from 78 to 94% at 1.1 kg/hectare and 76 to 93% at 11.1 kg/hectare. Photodieldrin and trans-aldrin diol were the major and minor conversion products identified, respectively. The chromatographic behavior of these two products matched the behavior of the reference standards. Three other minor conversion products were detected: products A, B and C. There was not sufficient quantities of any of the three to obtain a direct mass spectral analysis. Product A migrated between aldrin and dieldrin with the n-heptane:acetone (80:20) solvent system (R_f=0.64), behavior which

is similar to metabolite X reported by Klein et al. (1973). Product B migrated similar to metabolite C-1 of Matthews and Matsumura (1969) when developed in methylene chloride (R_f=0.19) and n-heptane:acetone (80:20) (R_f=0.34).These authors reported their metabolite C-1 to be a hydroxy photodieldrin metabolite. Product C migrated between trans aldrindiol and photodieldrin in n-heptane:acetone (80:20, R_f=0.18) and had a 0.05 R_f in methylene chloride. Polar materials remaining at the origin comprised 2-5% of the ^{14}C and were not identified.

The dieldrin EC formulation contained many impurities which were chlorinated and structurally similar to dieldrin. Many of the chlorinated impurities were absorbed by the plants along with dieldrin and were detected in the cleanup procedure as non-radioactive entities. The major impurity was chlorohydrin dieldrin.

Unextractable Radiocarbon. Amounts of unextractable ^{14}C residues, calculated as dieldrin equivalents, are shown in Table II for the 1.1 and 11.1 kg/hectare rates. Trends were toward increasing ppm bound over time. The percentages of recovered ^{14}C (extractable plus unextractable) that were bound also increased with time and were slightly higher from the 1.1 kg/hectare rate than from the 11.1 kg/hectare rate.

The bound ^{14}C residues were comprised of those releasable by hydrolytic treatments and those remaining lignin bound. Both total bound and lignin bound residues increased slowly post application through 14 days but increased rapidly between 14 and 21 days. The lignin bound ^{14}C ranged between 0.02 ppm to 0.07 ppm for both the 1.1 and 11.1 kg/hectare treatment rates at the 1, 7 and 14 day harvest periods. Between the 14 and 21 day harvest interval the lignin bound ^{14}C increased from 0.02 ppm to 0.18 ppm for the 1.1 kg/hectare rate and 0.07ppm

to 0.27 ppm for the 11.1 kg/hectare rate. An increase in lignin was also observed between 14 and 21 days.

An interesting observation was made which may suggest differences in extractability based on the mode of exposure, root absorption vs absorption through the leaves. If one compares data for the control and 11.1 kg/hectare application rate there were large differences in the percentage ^{14}C dieldrin that was unextractable. The control possessed 24-31% unextractable materials in the roots while the 11.1 kg/hectare treatment contained 3-24% unextractable ^{14}C. If one assumes that the control samples received the ^{14}C dieldrin predominantly as a leaf application resulting from volatilization of the insecticide(probably from the 111 kg/hectare application), and if one further assumes that the 11.1 kg/hectare rate received its dosage primarily as a root application, then differences in extractability appear to exist between the modes of exposure (or application). This cross-contamination assumption seems valid since the level of ^{14}C per gram of radish in the control was 10 to 20% of the ^{14}C levels in the 11.1 kg/hectare treatment.

The percentages of radiocarbon released from the extracted dry radish tissue by the various hydrolytic treatments are shown in Table V. The AOAC lignin analysis and 0.1 N HCl reflux released approximately the same amount of ^{14}C. This was approximately 2 times the amount released by the 0.1 N HCl + 1% pepsin digest at 40°C and approximately 3 times the amount released by 0.1 N HCl at 40°C alone. These values were obtained by combusting the extracted dry tissue before and after treatment.

The stability and extractability of dieldrin in 0.1 N HCl was evaluated to determine possible effects of the hydrolytic treatments on dieldrin residues. Technical dieldrin was spiked with ^{14}C dieldrin,

the mixture purified by TLC and added to 0.1 N HCl. One hundred percent of the ^{14}C would partition into methylene chloride both before and after refluxing. The methylene chloride fractions were then subjected to TLC and autoradiography. Before refluxing all the ^{14}C was dieldrin and after refluxing there were 12% conversion products.

The unextractable ^{14}C had several interesting characteristics. Seventy percent of the ^{14}C released from extracted tissue residue by the hydrolytic treatments remained in the aqueous phase when partitioned with methylene chloride whether the aqueous phase was acidic, neutral or basic. This polar ^{14}C material probably did not form as a result of the hydrolytic treatment since preliminary experiments using dieldrin as starting material showed it to partition into methylene chloride even after refluxing in 0.1 N HCl. The fact that adding pepsin to 0.1 N HCl increased the amount of ^{14}C released suggests the involvement of protein in some of the bound ^{14}C.

The ^{14}C remaining in the lignin fraction is probably covalently incorporated since it was not released or destroyed by the rigorous AOAC indirect procedure. This has been observed by other investigators for a variety of other pesticides in plants (Chin et al., 1973; Honeycutt and Adler, 1975; Forbes et al., 1980; Balba et al., 1979; Still et al., 1981; Khan, 1980; and Stratton et al., 1981). The nature of this complex is not known for dieldrin. However, studies with chloroaniline suggest covalent incorporation of the parent, or a structurally similar metabolite, into the lignin polymer (Still et al., 1981). Dieldrin does contain a reactive epoxide functional group which could be involved in an ether or ester covalent linkage.

More research is needed to identify the materials that can be released, to determine the structure of the "lignin-dieldrin" complex and to assess the toxicological significance of such unextractable residues.

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Table I. Hydrolytic Treatment Conditions

1. 0.1 N HCl, 40 C, 24 hours
2. 0.1 N HCl + 1% pepsin, 40 C, 24 hours
3. 0.1 N HCl, 100 C, 2 hours
4. AOAC Indirect Lignin Analysis[a]
   a. 0.1 N HCl + 1% pepsin, 40 C, 24 hours
   b. 5% H_2SO_4, 100 C, 1 hour
   c. 75% H_2SO_4, 20 C, 2 hours
   d. 3% H_2SO_4, 100 C, 1 hour

a. Performed on benzene:ethanol (2:1) extracted tissue

Table II. Concentration of ^{14}C as Dieldrin Equivalents at Time of Harvest

	1.1 kg/hectare				11.1 kg/hectare			
	1[a]	7	14	21	1	7	14	21
Extractable	1.92	1.09	0.29	0.85	8.27	7.10	3.88	3.12
Unextractable[b]	0.15	0.31	0.11	0.54	0.23	0.23	0.56	0.96
Total[b]	2.10	1.40	0.40	1.39	8.50	7.33	4.44	4.08

[a] Days post application

[b] Average of two combustion analyses

Table III. Percentage Recovery of Applied Radiocarbon from Radish Roots for the 1.1 and 11.1 kg/hectare Treatment Rates at 1, 7, 14 and 21 Days.

Treatment Rate (kg/hectare)	Day Post Application			
	1	7	14	21
1.1	1.6	3.6	2.2	7.6
11.1	0.6	1.2	1.4	2.3

Table IV. Percentage Distribution of C-14 in the Extractable Fraction as Dieldrin and Conversion Products

	1.1 kg/hectare				11.1 kg/hectare			
	1	7	14	21	1	7	14	21
Dieldrin	93.8	89.6	78.2	80.4	93.4	88.9	76.5	78.9
Photodieldrin	3.3	5.0	7.4	8.0	2.7	5.0	10.4	9.3
trans-aldrindiol	0.0	0.0	3.4	1.0	0.0	0.0	1.8	1.2
A [a]	0.0	1.0	1.0	1.0	0.0	1.0	2.0	2.0
B [a]	0.0	0.5	1.2	1.2	0.0	1.5	2.0	2.0
C [a]	0.0	2.0	3.0	4.0	0.0	0.5	2.3	2.0
Polar [b]	2.7	1.9	5.8	4.4	3.9	3.1	5.0	4.6

[a] Unknown conversion products, see extractable Radiocarbon section for chromatographic behavior.

[b] Radiocarbon remaining at the origin

Table V. Percentage Radiocarbon Released from the Solvent Extracted Dry Tissue by the Hydrolytic Treatments

	1.1 kg/hectare				11.1 kg/hectare			
	1[a]	7	14	21	1	7	14	21
0.1 N HCl, 40°C[c]	-[b]	-	19.2	11.8	-	-	17.1	20.6
0.1 N HCl+1% pepsin, 40°C[c]	-	-	32.4	34.4	-	-	36.5	33.6
0.1 N HCl, 100°C[c]	-	-	64.2	76.7	-	-	75.9	84.1
Lignin Analysis[d]	88.1	86.3	80.7	66.5	92.9	92.5	86.7	71.8

[a] Days post application

[b] These treatments were not performed on the 1 and 7 day tissues

[c] Average of duplicate determinations

[d] Average of triplicate determinations

BOUND RESIDUES OF ^{14}C-LINDANE IN RICE PLANTS AND SOIL

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Abstract

The extractable ^{14}C-residues decreased with time in both unflooded (upland) and flooded (low land) ^{14}C-gamma HCH treated soils. In both unflooded and flooded soils, considerable amounts of ^{14}C-bound residues were formed. Partially bound residues could be detected after 90 and 120 days in unflooded soils. A considerable amount was detected as volatile residues. Addition of green manure lowered bound residue formation and volatilization. The ^{14}C-residues were incorporated in fulvic and humic acid fractions of soil organic matter. Although no significant residues were left in rice, husk or straw samples of ^{14}C-HCH treated rice plants, the bound residue formation in rice straw may become important if bioavailable.

INTRODUCTION

1,2,3,4,5,6-hexachlorocyclohexane (HCH) is the most widely used pesticide in India (50% of the total consumption). Several studies were carried out on the fate and persistence of lindane (gamma-HCH) in soils and the magnitude of extractable residues in rice and straw. However, data on the formation of bound residues in plants and soils are lacking. This report gives some information on bound residues of ^{14}C-lindane in soil and rice plants.

MATERIALS AND METHODS

Soil studies: Ten g black clay loam soil (pH 7.8; organic C, 0.92%) samples were treated with ^{14}C-gamma-HCH (sp. activity 48 mCi/mmol.) at a concentration of 1 mg/kg of soil and incubated under flooded and unflooded conditions. Three replicates were maintained for each treatment. Polyurethane plugs were used to trap any volatilized residues. The tubes containing soil were kept in large porcelain pots

containing soil under flooded and unflooded conditions to avoid drastic changes in the external temperature. The pots in turn were kept outdoors to simulate field conditions. Green manure (leaves of Glyricidia sepium) was added to the tubes at a rate of 11230 kg ha^{-1} and ploughed in with the help of a glass rod after 7 days of flooding. Samples were taken after 30, 60, 90 and 120 days.

The soils were extracted twice with acetone for a period of 30 min. and then additional extractions with benzene:methanol:acetone (1:1:1) mixture (BMA) for a period of 30 min. till no ^{14}C-residues were recovered. At each step, the extracts were filtered through Whatman filter paper No. 42, using a Buchner funnel.

The soil samples after solvent extraction were air-dried and combusted in a Harvey Biological Material Oxidizer which gave 85% recovery of the added ^{14}C-pesticide in the form of $^{14}CO_2$. Since the liquid scintillation cocktail for CO_2 estimation showed quenching, corrections were made to compare it with cocktail D (dioxane, naphthalene and PPO mixture) which was used for counting soluble or extractable radioactivity. The radioactivity was determined by a Packard liquid scintillation counter and quench corrections were made by the external standard mode.

The unextractable residues, bound to fulvic and humic acid constituents, were determined as described by U.S. EPA [1].

Volatile ^{14}C-residues were determined by extracting the polyurethane plugs with acetone:hexane (1:1) in a Soxhlet apparatus for 6 hrs. and the ^{14}C-content was quantitated by liquid scintillation counting [2].

For determining the release of ^{14}C-bound residues, the extracted soils (flooded and unflooded) were mixed with fresh soils (4 g of soil containing bound residues + 6 g of fresh soil). One set of tubes was autoclaved. Sodium azide (1%) was added to sterilize soils to prevent any possible contamination from the extracted soil. The soils were incubated at 30°C for 18 days and extracted as previously described.

Plant studies: Rice plants (TR 17 variety) were grown in porcelain pots containing 15 kg of black clay loam flooded soil and kept under outdoor conditions. Two plants per pot were maintained. ^{14}C-gamma-HCH (sp. activity 82.7 μCi/μmole) was mixed with technical HCH and sprayed at the agricultural application rate of 4.86 kg of 5% HCH dust per ha to rice plants before the onset of flowering. The volume of spray was adjusted to form a coating of dust immediately. Plants without ^{14}C-HCH treatment served as control.

The rice plants were harvested at maturity and dried in the sun. The panicles and straw were separated. The rice, husk and straw were homogenized in a power mill and extracted with methanol for 36 hrs. in a Soxhlet apparatus. The radioactivity in the extract was counted and quench corrections were made by the internal standard method. The unextractable ^{14}C in various samples was measured in a liquid scintillation counter after oxidation to $^{14}CO_2$ in a Biological Material Oxidizer.

RESULTS AND DICUSSION

At '0' day, 99% of the ^{14}C-lindane residues could be extracted in the first acetone extraction and no bound residues were detected. The percent distribution of ^{14}C-residues in flooded (lowland) and unflooded (upland) soils is given in Table I. The percent extractable residues decreases more in flooded than in unflooded soils over a period of 120 days. It is known that gamma-HCH is rapidly biodegraded under flooded conditions from experiments on the persistence of gamma-HCH in sterilized and unsterilized soils, on isolation of Clostridium spp. and on $^{14}CO_2$ evolution from ^{14}C-lindane treated soils [3, 4, 5, 6].

A considerable amount of ^{14}C-HCH was lost from soil due to volatilization in both unflooded and flooded soils. Lindane is known to volatilize rapidly due to its high vapour pressure [3, 7], and this is demonstrated in this experiment.

The present results indicate that bound residues are significant in ^{14}C-HCH treated soils. In unflooded soil, the bound residues appear to increase with time and there is a good total recovery of added lindane. The magnitude of residues is in the range reported for other persistent insecticides [8]. However, a large percentage of the added HCH could not

be accounted for in the experiment conducted in flooded soil, possibly related to the formation of $^{14}CO_2$, ^{14}C-methane or ^{14}C-benzene, etc.

Interestingly, in flooded soils considerable bound residues are also formed (Table I). At the end of one month 28% of the added radioactivity was present as bound residues. Since lindane is known to be degraded in flooded soils, it is possible that the degradation products (e.g. chlorophenols) increase with time and become bound. Khan and Ivarson [9] have also suggested that degradation products of ^{14}C-prometryn become bound to soil. In flooded and unflooded soil samples (30 and 60 days), two acetone and three BM extractions were necessary to recover all extractable ^{14}C-activity. However, in case of 90 and 120 days unflooded soil samples, three additional BM extractions were required to remove the total extractable radioactivity. This suggests the possibility of formation of partially bound residues. Helling and Krivonak [10] have observed a similar phenomenon with dinitroaniline residues.

Green manuring is an agricultural practice in rice fields, and we have recently shown rapid loss of the isomers of HCH in soil by green manure amendment [11]. It was of interest to see the effect of this practice on the build-up of bound residues in soil. Figure 1 shows that bound residues were less in amended than in unamended flooded soil. This tendency was also observed with volatile residues. These results clearly demonstrate the potentiality of using green manure for the decontamination of pesticide-polluted soils, as there was no additional build-up of bound residues.

Table II shows the distribution of ^{14}C-residues in soil organic matter constituents. Several workers have shown the distribution of bound ^{14}C-residues among humic fractions of soil treated with ^{14}C-pesticides [10, 12, 13]. Khan [14] reported the presence of bound residues in the fulvic acid (FA) fraction under field conditions and their possible bioavailability to both plants and soil fauna.

The release of bound residues by microorganisms has been reported by Khan and Ivarson [9]. In the present paper, some bound residues appear

to be released after 90 and 120 days in unflooded soil. To verify this, studies were conducted on the release of ^{14}C-residues from soil containing ^{14}C-bound residues mixed with fresh soil under unsterilized and sterilized, flooded and unflooded conditions (Table III). No difference was observed between sterilized and unsterilized treatments, but it is significant that more residues were released in unflooded than flooded soils.

^{14}C-residues in rice, husk and straw samples were small (Table IV). The straw samples tend to have more bound than extractable residues. As straw is used as cattle feed and also for mushroom cultivation, the bioavailability of these residues may be significant.

It is likely that these residues are associated with the lignin fraction of straw, for in plants treated with nitrofen or azinophos the ^{14}C from the pesticides was incorporated into the lignin fraction [15, 16]. The soils which supported the growth of rice plants contained more bound than extractable residues.

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Table I. Percent distribution of ^{14}C-lindane in black clay loam flooded and unflooded soil

	Percent ^{14}C-residues			
	Incubation period (days)			
	30	60	90	120
UNFLOODED				
Extractable	59.35	45.38	39.84	42.84
Volatiles	15.01	15.82	16.74	20.21
Bound	19.52	23.67	27.37	29.45
Total	93.88	84.87	83.95	92.50
FLOODED				
Extractable	28.29	11.78	7.90	5.43
Volatiles	16.63	15.94	16.97	18.59
Bound	27.71	20.55	18.48	24.13
Total	72.63	48.27	43.35	48.15

Table II. Bound residues of ^{14}C-lindane in fulvic and humic acid fractions of soil

	Percent ^{14}C in bound residues							
	Fulvic acid				Humic acid			
Treatment	30	60	90 (days)	120	30	60	90 (days)	120
Unflooded	13.13 (2.56)	9.05 (2.14)	6.57 (1.80)	9.05 (2.66)	2.23 (0.43)	1.59 (0.38)	1.32 (0.36)	1.39 (0.41)
Flooded	13.66 (3.79)	17.80 (3.66)	15.45 (2.85)	13.78 (3.33)	1.20 (0.33)	3.40 (0.70)	4.41 (0.81)	2.76 (0.67)
Green manure	20.68 (1.96)	15.18 (1.82)	27.93 (2.10)	16.12 (1.41)	5.41 (0.51)	3.36 (0.40)	4.72 (0.36)	3.61 (0.32)

Numbers in parentheses represent percentages of original radioactivity.

Table III. Release of ^{14}C-residues from soil-bound residues

	Percent ^{14}C-bound residues released (^{14}C-bound residues = 100%)	
	Extractable	Bound residue
UNFLOODED		
Unsterilized	32.70	61.05
Sterilized	36.74	53.72
FLOODED		
Unsterilized	15.94	66.54
Sterilized	19.12	79.60

Table IV. ^{14}C-residues in rice, husk and in paddy soil

	^{14}C-residues (µg/g) (equivalent to HCH)		
	Extractable	Bound	Total *
Straw	0.019	0.038	0.060
Rice	0.009	0.002	0.012
Husk	0.015	0.003	0.020
Soil	0.02	0.10	0.12

*Determined by combustion of the unextracted sample.

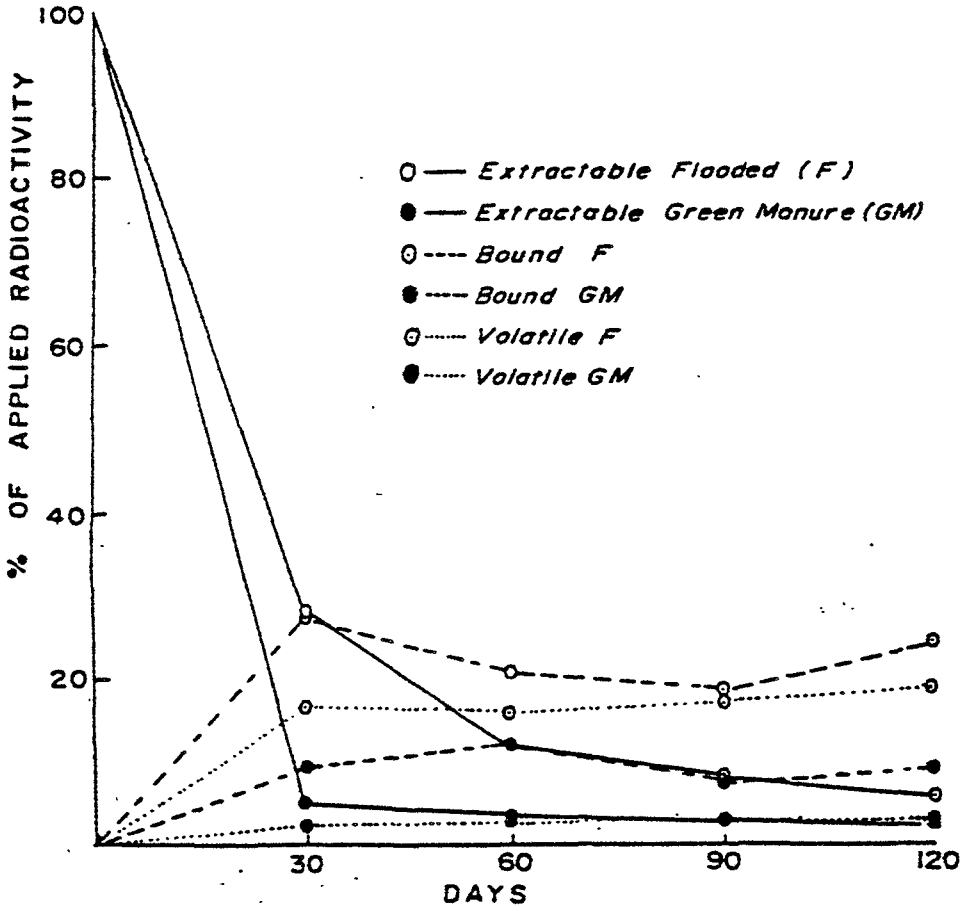

FIG.1 EXTRACTABLE, BOUND AND VOLATILE RADIOACTIVITY IN SOIL TREATED WITH ^{14}C-LINDANE.

RESIDUES OF ENDOSULFAN IN TOMATO

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Abstract

The fate of endosulfan in tomato leaves, fruits and its uptake by seedlings from nutrient solution was studied using ^{14}C-endosulfan ($\alpha+\beta$). The compound was found to disappear rapidly from leaf surface with a half-life of 6-7 days. Approximately 20% of the applied activity was detected inside the leaves and was extracted most effectively with hexane/ethanol mixture. Endosulfan sulfate and endosulfan alcohol were found only in the internal leaf extracts. On incubating α- and β-isomers separately in ripening on-plant tomato fruits for 24 hr., over 95% of the radioactivity was recovered by methanol extraction. However, about 3% of radioactivity remained non-extractable in the cake. Uptake of radioactive endosulfan by seedlings over a 4-day period reached up to 7% of activity in the medium.

INTRODUCTION

Vegetable production is becoming an increasingly important part of the Sudan Gezira cropping system. About 40,000 feddan (1 feddan = 0.42 ha) are devoted to vegetable cultivation [1], mostly tomato, because of its high economic returns on both local and foreign markets.

Tomato production is, however, adversely affected by attacks of pests and diseases which require efficient chemical control measures. The insecticide endosulfan at a rate of 0.8 lb/feddan is widely used against whiteflies and other minor pests [2, 3] such as the bollworm Heliothis armigera (Hb).

Previous studies on the persistence of endosulfan in tomato under Sudan conditions have demonstrated its rapid disappearance with a half-life of 1-2 days [4], and the compound was considered reasonably safe when used at the recommended dose. Gas-liquid chromatography was the main analytical method employed during these investigations.

Endosulfan is known to undergo a number of transformations under various environmental conditions [5]. Products of such conversions include the dihydroxy derivative, the α-hydroxy ether, the lactone and the sulfate. Some of these compounds have the potential to be conjugated or bound within the plant. Such possibilities, in addition to the demonstrated ability of endosulfan to penetrate the tomato fruit cuticle [4], show clearly the limitations of residue data which are based on extractable, GLC measurable residues.

The present set of experiments was conducted using ^{14}C-labelled endosulfan in order to elucidate with better precision the important aspects of the fate of endosulfan on an important crop.

MATERIAL AND METHODS

Endosulfan (α+β) 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzadiexathiepin-3-oxide-(5a,C9a-^{14}C), 100 mg (0.100 mCi) was supplied by Hoechst AG through Bittar Co. Sudan. The material had 98% radiochemical purity as certified by Hoechst Radiochemical Laboratory. The specific activities and purity of materials prepared from this stock were determined by liquid scintillation counting, GLC and TLC in our laboratory.

Samples of endosulfan isomers and metabolites from Riedel-de-Haen, Hannover were also supplied by Bittar Co. Liquid scintillation counting was carried out using a Packard Tri-Carb model 300 CD instrument. Aliquots of samples were mixed with Packard Insta-Gel, xylene based cocktail for aqueous and non-aqueous samples. Beckman Tissue Solubiliser-450 was used to treat tomato fruit tissue (1 ml/200 mg).

Thin-layer chromatography was performed on glass plates, using silica gel and silica gel GF 254 as adsorbents. The developing system was a mixture of hexane/acetone (6:1). Autoradiography of TLC chromatograms and plants was carried out using Kodak X-ray film SB-5, Kodak GBX developer and Replenisher and Kodak Rapid Fixer.

Gas-liquid chromatography was performed on two instruments, a Perkin-Elmer F 11 with an election capture detector and a Tracor 560 GLC. Stationary phases were Dexil 300 1 % and 3 % QF 1 - SE 30 (1:1) on

chromosorb W 80-100 mesh. All solvents and chemicals were analytical grade reagents.

Persistence in Tomato Leaves

^{14}C-endosulfan ($\alpha+\beta$) was applied to the upper surface of selected leaves in healthy field grown tomato plants. The amount of activity per leaf was approx. 4000 dpm. Samples (5 leaves) were collected after the first hour and then at 24 hr. intervals for seven days. External residues were recovered by washing leaves in benzene, 3 x 10 ml. Internal residues were extracted by two procedures, the conventional hexane/ethanol and by maceration in methanol.

Portions of extracts were counted and the remainder was rotovaporated and examined by TLC:

Fate in Tomato Fruits

The two endosulfan isomers were separated and purified by TLC and autoradiography. Sets of four fruits were injected with 25 µl of a benzene solution of each isomer, at a rate of 400 x 10^3 dpm per fruit for α-endosulfan and of 150 x 10^3 dpm per fruit for β-endosulfan.

After 24 hr., the fruits were collected, chopped and blended with methanol (300 ml). After contrifugation, an aliquot of the methanol extract was counted. To determine the non-extractable activity, portions of the extracted tissues (200 mg) were treated with 2 ml Beckman tissue solubiliser-540 (0.5 N quaternary ammonium hydroxide in toluene) at 40° for 24 hr. prior to counting.

Methanol extracts were chromatographed on Florisil and analysed by GLC.

Uptake by Tomato Seedlings

^{14}C-endosulfan ($\alpha+\beta$) was incorporated in a plant nutrient medium at 5000 dpm/ml in a growth plate fitted with a perforated black cover. Three sets of 4 tomato seedlings 1 month old were placed in such plates.

A fourth plate with a similar set of plants, but containing no endosulfan served as control. Plants were fitted in such a way that only root systems were dipped in solution. Plates were sampled at 24, 72 and 96 hr. intervals. Radioactivity in plants and medium was counted.

RESULTS AND DISCUSSION

The radioactivity recovered from external wash and internal leaf extractions expressed as a percentage of the amount initially applied is shown in Table 1. Table 2 summarizes the nature of endosulfan products as determined by TLC and GLC.

The results indicate a half-life of 6-7 days for endosulfan in tomato leaves. This is considerably longer than has been reported earlier from experiments under Sudan conditions [6]. It might be attributed primarily to the cold weather which prevailed during the time of this experiment (10-25°) as compared to the summer conditions (30-42°) of the previous study. In contrast to the findings of Harrison et al. [7], endosulfan sulfate was not detected in surface washings. Its absence was previously noted from similar experiments on cotton plants [8]. It seems likely that the formation of the sulfate, an important terminal residue of endosulfan takes place by an enzymatic process. This is indirectly supported by the work of Archer et al. [9] which reported the absence of sulfate after 7 days of ultra-violet irradiation of endosulfan on a glass surface.

After incubation of the α- and β-isomers with the fruit for 24 hr., most of the radioactivity could be recovered by methanol extraction (Table 3). A small fraction, approximately 3%, was non-extractable as measured by LSC after solubilization of the extracted tissue. By Florisil chromatography of the methanol extracts, a loss of radioactivity was observed amounting to 30% and 40% for the α- and β-isomers, respectively. This suggests possible formation of polar derivatives not elutable by the 15% ether hexane from Florisil. GLC analysis showed the presence of the unchanged isomers only.

The uptake of radioactive endosulfan increased over a 4-day period only to a maximum of about 7% of the applied dose. This is consistent

with the expected behaviour of a non-systemic, highly active lipophilic organochlorine compound. Loss of activity from the nutrient medium was fast and is probably due to volatilization and co-distillation with water (Table 4).

The presence of endosulfan in the plant material was also confirmed by TLC.

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Table 1 - Persistence of ^{14}C-endosulfan in Tomato Leaves

Time after application	% Activity		
	External	Internal methanol	Hexane/ Ethanol
1 hr.	70	7.7	9.6
1 day	63	11.8	9.8
2 days	55	18.6	17.3
3 "	53	17.3	23.0
4 "	41	18.7	27.0
5 "	33	13.2	26.0
6 "	26	12.0	18.0
7 "	25	8.5	15.6

Table 2 - Residues of Endosulfan and Products in Tomato Leaves

Compound	Extract	
	External	Internal
α-endosulfan	+	+
β-endosulfan	+	+
endosulfan ether	+	-
endosulfan sulfate	-	+
endosulfan alcohol	-	+

Table 3 - Fate of Isomers in Tomato Fruits

Treatment	% Radioactivity		Ether-hexane elutable activity from Florisil
	Methanol Extract	Extracted Tissue	
α-isomer	96.0	3.0	70 %
β-isomer	98.0	3.0	60 %

Table 4 - Uptake of Endosulfan by Tomato Seedlings

Days	% Activity	
	Plant	Medium
1	3.4	55.8
3	4.5	47.0
4	6.9	46.0

PERSISTENCE OF ATRAZINE AND ITS METABOLITES IN SOIL EIGHT YEARS AFTER A SINGLE HERBICIDE APPLICATION AND THEIR UPTAKE BY OAT PLANTS

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Abstract

The fate of atrazine in soil was investigated under field conditions. In spring 1973 the soil was treated with ^{14}C-ringlabeled atrazine and cultivated with various plant species each year. In summer 1981 soil samples were collected and analysed. The soil still contained about 83 % of the total initially applied ^{14}C-activity. From this only 40 % could be extracted and analysed. Besides traces of atrazine six metabolites were further identified which originated from the parent compound through N-dealkylation and hydrolysis. The soil containing only bound (non extractable) ^{14}C-residues was fractionated into humin, humic acid and fulvic acid fractions. The distribution of the residual ^{14}C-activity was: 54 %, 11 % and 35 %, respectively.

The soil in the culture boxes was sowed with oats in May of 1982. In August the oats were harvested and analysed. The uptake amounted to 0.03 % of the total initially applied ^{14}C-activity. 2-Hydroxy-4-amino-6-isopropylamino-s-triazine and 2-hydroxy-4-ethylamino-6-amino-s-triazine were identified in conjugated form in the extractable part.

Introduction

Bound pesticide residues are basically those residues remaining in soil or plant after exhaustive solvent extraction, so they cannot be detected in the routine analysis.

They have been the subject of numerous investigations which suggest that bound residues of pesticides are not excluded from environmental interactions. A number of recent studies has demonstrated the potential availability of bound pesticide residues to plants (Führ and Mittel-

staedt, 1980; Führemann and Lichtenstein, 1978; Helling and Krivonak, 1978; Khan, 1980; Süß and Grampp, 1973), to earthworms (Führemann and Lichtenstein, 1978), their biodegradation by soil microbes (Khan and Ivarson, 1981), and their distribution among the soil organic matter fractions (Khan, 1982).

Khan and Hamilton (1980) have developed a high temperature distillation technique for determining and chemically identifying the bound residues in soil and plant material. Contrary to the general belief that bound residues become an integral part of the polymolecular structure of the soil organic matter without a recognizable relationship to the original pesticide Khan's results unequivocally demonstrate that the bound residues comprise a significant amount of the parent compound. Thus it is conceivable that in addition to chemical binding the physical binding may also play an important role in the formation of bound residues.

According to these facts the bound pesticide residues in soil could represent a potential hazard for the environment, assuming that the parent compound or one of its phytotoxic metabolites would be released by one of the processes mentioned above. So, more intensive investigation is necessary to understand the mechanism of binding between pesticide residues and soil matrix.

Among other herbicides the triazine compounds are of great significance as herbicide active substances. An experiment in which the soil was treated once with ^{14}C-ringlabeled atrazine (2-Chloro-4-ethylamino-6-isopropylamino-s-triazine) was started 1973 in our laboratory (Süß, 1978). In 1981 we established that the contaminated soil still contained a considerable amount of the initially applied radioactivity.

The aim of our study is:

- to establish the chemical nature of the extractable radioactivity
- to investigate the plant availability of the radioactivity present in soil long time after the herbicide application
- to determine the distribution of the bound (non extractable) radioactivity among the soil organic matter fractions

- to elucidate the mechanism of the binding between herbicide and humic matter
- to examine the accumulation of residues in soil after a consecutive annual atrazine application

The data reported herein are a compilation of our results obtained during the last two years (Capriel and Haisch, 1983).

Material and Methods

1. Experimental Set-up

We investigated a brown soil having the following physico-chemical characteristics of the vegetable mould: pH = 5.3 ($CaCl_2$), 2.88 % C, 0.33 % N, C/N 8.7, 8.2 % clay, 14.9 % silt, 76.9 % sand (Süß, 1978).

The experiment was conducted under field conditions. The culture boxes were buried into the soil and filled layer by layer with the naturally grown soil. The boxes were provided with holes to permit the drainage. In the spring of 1973 the surface area of the soil in each box was sprayed with a solution containing ^{14}C-ringlabeled atrazine (170.6 mg m^{-2}, spec. activ. 17.2 μCi mg^{-1}; Süß, 1978). Between 1973 and 1981 the following plants were cultivated: maize (1974, 1976, 1978 - 1981), summer wheat (1975), cabbage and carots (1977). In summer 1981 soil samples were collected from the 0 to 15 cm layer. These were air dried, screened, passed through a 1 mm sieve, homogenized and stored in sealed plastic bags at - 20 $^{\circ}$ C until they were analyzed. In early May of 1982 the soil was sowed with oats. Oats were chosen as they do not readily degrade s-triazine (Ashton and Crafts, 1973). In August the oat plants (straw and grains) were harvested, freeze-dried, finely ground, homogenized, and stored in sealed plastic bags at - 20 $^{\circ}$ C until they were analyzed.

2. Apparatus and chemicals

GC Varian 1420 fitted with a Grob split-splitless injector and an AFID having a Rb_2SO_4 annulus; Carriergas: helium; Fused silica WCOT 25 x 0.32 mm i.d. coated with SE 54 (0.2 μm);

Fused silica WCOT 25 x 0.32 mm i.d. coated with Carbowax 20 M (0.2 .m); Intertechnique Sample Oxidizer (Model IN 4101); Beckman Liquid scintillation counter (Model LS 8500); Berthold TLC Scanner (Model 2723).

All solvents used were of analytical grade and were redistilled. For the TLC we utilized HPTLC silica gel plates, 60 F 254, Merck. They were prewashed with ethylacetate - methanol 1 : 1 (v/v) and activated at 110 ^{o} C for 30 min. The diazomethane was freshly prepared before using. The reference standards of atrazine and metabolites were gifts from Ciba Geigy Ltd., Basel, Switzerland.

3. Analytical part

3.1 Soil

Fifty grams aliquots of air dried soil were placed in the glass insert of a hot extractor and were exhaustively extracted with methanol under reflux. The work-up of the methanol extract containing the extractable radioactivity was analogous to that described by Khan et al. (1977).

The extracted soil sample which contained only the bound (non-extractable) residues was further fractionated in order to determine the distribution of the residual ^{14}C among the soil organic matter fractions.

The method of extraction, separation and purification of humic materials outlined in Fig. 1 is identical to that described by Matsuda and Schnitzer (1972).

3.2 Plant

The analytical procedure outlined in Fig. 2 was used for the extraction and the clean-up of the plant material, and it was similar to that employed by Khan et al. (1977, 1980). The extraction was carried out in an ultra-sound bath.

Results and discussion

Capillary GC analysis of extracts of experimental samples showed peaks having retention times identical with those of the reference standards. The chemical identity of the peaks was confirmed by co-chromatography with authentic standards, by TLC and scanning by

comparing the R_F values of the ^{14}C radioactive bands with those of the standards and finally by mass spectroscopy.

The efficiency of methylation of the 2-hydroxy metabolites with diazomethane was about 40 % in accordance with the literature data. Thus, the values reported for residues of hydroxy metabolites should only be regarded as qualitative.

The soil still contained about 83 % of the 1973 applied ^{14}C activity, as determined by combustion to $^{14}CO_2$ and liquid scintillation counting. From this only 40 % could be extracted and analyzed (Tab. 1).

Tab. 1: Residues of atrazine and its metabolites in the soil extract

Compound	Concentration [a, b, c] $ng \cdot g^{-1}$
Atrazine	0.9 ± 0.3
2-Chloro-4-amino-6-isopropylamino-s-triazine	8 ± 1.5
2-Chloro-4-ethylamino-6-amino-s-triazine	<0.2
2-Chloro-4,6-diamino-s-triazine	11 ± 1.2
Hydroxyatrazine	110
2-Hydroxy-4-amino-6-isopropylamino-s-triazine	18
2-Hydroxy-4-ethylamino-6-amino-s-triazine	10

[a] air-dry basis

[b] mean value with standard error

[c] values are not corrected for recovery

It is surprising that traces of atrazine were still present in soil eight years after the herbicide application. The relative large concentration of 2-chloro-4,6-diamino-s-triazine is also worth mentioning, because the degradation of this metabolite in soil has been found to occur very rapidly (Wolf and Martin, 1975).

Its concentration is of the same order of magnitude as that of deethylatrazine. The presence of residues of metabolites containing both 2-chloro- and 2-hydroxy moieties indicates that both hydrolysis and N-dealkylation were involved in the metabolism of atrazine in soil.

The soil containing only bound (non extractable) ^{14}C-labeled residues was fractionated into humin, humic acid and fulvic acid fractions. The distribution of the residual ^{14}C-activity was: 54 %, 11 % and 35 %, respectively. The incorporation of ^{14}C bound residues into humic materials and their distribution observed in our study is consistent with that reported by Khan (1982) for prometryn. The presence of ^{14}C bound residues in the FA fraction (35 %) is of special interest, with regard to their bioavailability, because the FA fraction is considered to be the dominant soluble organic fraction present in the soil solution under field conditions (Khan, 1982).

Of course the question arises whether the ^{14}C bound activity is still present as an intact triazine ring. There is evidence that the s-triazine ring structure is somewhat resistent to microbial degradation. In nearly all experiments only low levels of $^{14}CO_2$ have been evolved from microbial systems treated with ring labeled s-triazines (Kaufman and Kearney, 1970; Esser et al., 1975). Since little or no correlation has been observed between herbicide degradation and carbon dioxide evolution, it seems likely that complete ring cleavage is not a major mechanism of s-triazine degradation in soil.

The occurence of a slow rate of break-down might be due to the effects of adsorption - desorption equilibria on availability of the s-triazine for uptake and metabolism by soil microorganisms. In other words the rate limiting process could be of physical or chemical nature rather than biological.

At this time we can not answer the above question, but work is in progress in our laboratory.

The investigated plant material (straw and grains) contained about 0.03 % of the initially applied ^{14}C-radioactivity. From this 90 % were included in straw and 10 % in grains. The low assimilation is

consistent with the experimental results of Süß (1978). From the ^{14}C activity comprised in plant only 75 % could be extracted and analyzed (Tab. 2). The rest (25 %) can be considered as bound residues.

Tab. 2: Hydroxy metabolites of atrazine in oat straw and grains, respectively

Plant part	Concentration [a, b] $ng \cdot g^{-1}$	
	2-Hydroxy-4-amino-6-isopropylamino-s-triazine	2-Hydroxy-4-ethylamino-6-amino-s-triazine
Straw	12	8
Grains	<0.2	-

[a] freeze-dry basis

[b] values not corrected for recovery

The identified metabolites were all in the conjugated form and could be released and analyzed only after hydrolysis. In this study no attempt was made to determine the nature of the conjugates.

Neither free 2-chloro- nor 2-hydroxy-metabolites could be found in the plant extracts. It is also of interest to note that we could not find any free or conjugated hydroxyatrazine, although its concentration in soil was larger compared to the other metabolites. It seems likely that the hydrcxyatrazine was absorbed and metabolised via N-dealkylation and conjugation.

The results of this study show that a significant amount cf residues can persist in soil under field conditions even long time after a single atrazine application. In spite of this their plant availability seems to be very small campared to the quantity of extractable residues. Hydroxyatrazine and its monodealkylated analogues represent the main metabolites in the soil extract. Furthermore, we indentified traces of atrazine and its mono and completely dealkylated degradation products. In the plant extracts we could find only monodealkylated 2-hydroxy-metabolites in the conjugated form.

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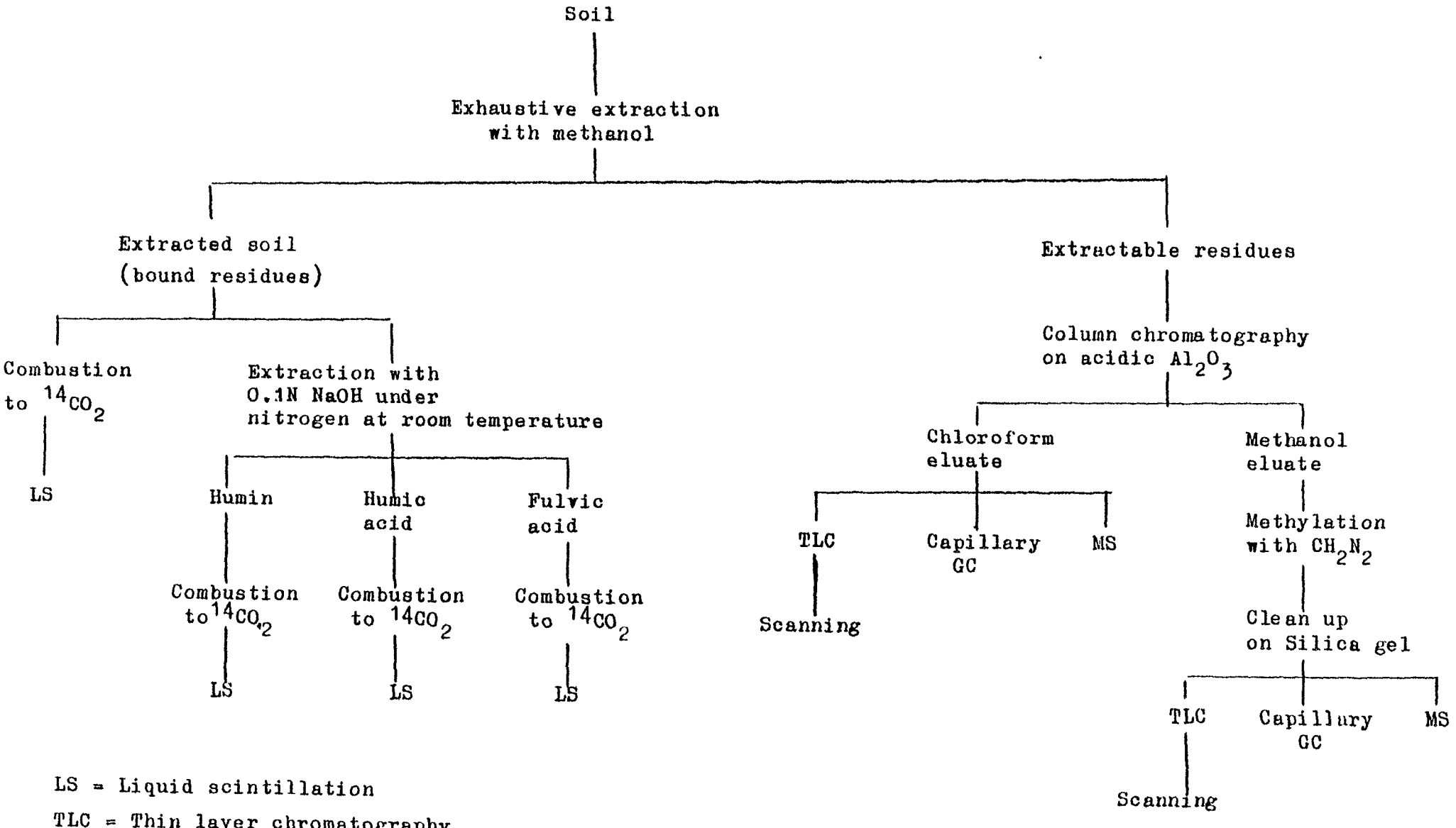

LS = Liquid scintillation

TLC = Thin layer chromatography

Fig. 1: Flow diagram of the fractionation and analysis of soil containing ^{14}C labeled residues

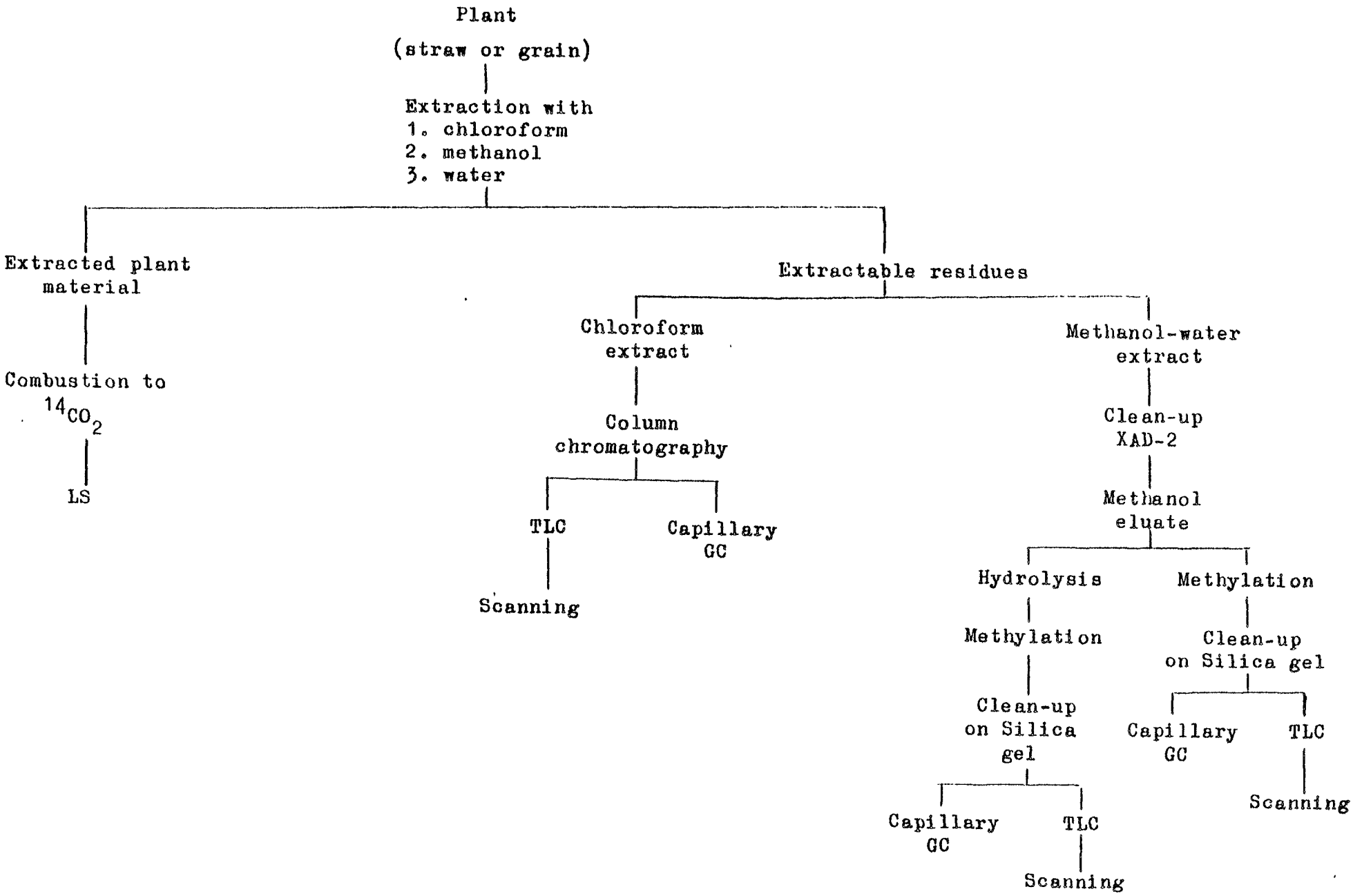

Fig. 2: Diagram of the fractionation and analysis of plant material

FORMATION OF BOUND RESIDUES OF (RING-^{14}C) SIMAZINE IN A PARABRAUNERDE (ALFISOL) AND THEIR AVAILABILITY TO MAIZE

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Abstract

(Ring-^{14}C) simazine was incubated in a Parabraunerde (Alfisol) at 22 —C and 65 % of the maximum water holding capacity of the soil for 108 days. The $^{14}CO_2$-evolution was registered and soil samples were extracted at day 1, 37, 78 and 108 employing 0,2 M ascorbic acid, 0,2 M citric acid and acetone (1:1:2) to study the metabolite spectra and the formation of bound residues in the soil.

In specially developed experimental pots the bioavailability of the bound residue fraction was studied using maize plants. Within 30 days 20 % of the applied radioactivity was taken up via the roots and predominantly translocated to the shoots when /ring-^{14}C/ simazine was added to the soil immediately before the plant experiment. This radioactivity fraction decreased to 10 % when the maize was grown in soil containing aged simazine residues after 108 days of degradation. If the soil contained only bound residues, produced as described above, only traces of radioactivity (0,08 % of the applied radioactivity) became bioavailable to roots. A desorption study with water reflected the relative bioavailability of the residues in the soil.

INTRODUCTION

The ideal pesticide active ingredient should be degraded into its basic structural elements after it has been applied to the soil or plant. However, in practice work with ^{14}C-labelled active substances had indicated that with increasing residence time and rising soil temperature the fraction of non-extractable radioactivity, which thus cannot be characterized by chemical analyses, increases in the soil (Führ and Mittelstaedt, 1976; Lichtenstein, Katan and Anderegg, 1977; Führ and Mittelstaedt, 1979). Pesticides thus behave in the same way as other, natural organic compounds in the soil (e.g. straw, basal dressing, roots), which contribute to the structure of the organic mass of the soil during the degradation processes (Sauerbeck and Führ, 1970, 1971; Stevenson, 1976; Oberländer and Roth, 1980). The compounds of the organic matter in the soil have reactive groups which can form strong bonds with pesticide active substances or their metabolites. These residues can then no longer be extracted by means of conventional extraction procedures.

Since the mid-seventies, this fraction of the pesticide residues in the soil has been increasingly investigated more intensively and systematically. In this connection it is clear that the non-extractable residues in plants (Süß and Grampp, 1973; Fuhremann and Lichtenstein, 1978; Helling and Krivonak, 1978; Haque et al., 1981; Führ and Mittelstaedt, 1980; Khan, 1980), earthworms (Fuhremann and Lichtenstein, 1978; Haque et al., 1981) and microorganisms (Khan and Ivarson, 1981, 1982) can become biologically available so that radioactivity can be taken up by organisms and in individual cases the initial active substance has been identified (Führ and Mittelstaedt, 1980).

Until now chloroanilines, phosphorus esters and urea derivatives have been the most investigated. In addition, triazine compounds are also of great significance as herbicides. Some representatives are particularly characterized by relative stability in the soil. For this reason we selected a representative of the chlorinated s-triazines, simazine.

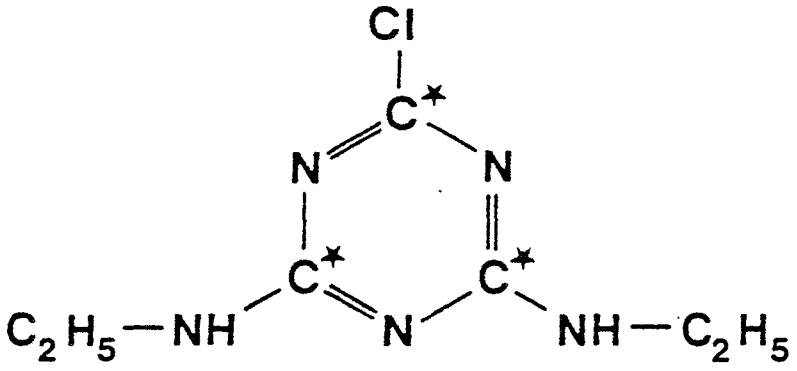

Fig. 1: Structural formula and labelling position (*) of Simazine.

First of all, the formation of bound residues was observed after application of ring-labelled ^{14}C simazine in a Parabraunerde. Based on this, the root uptake of ^{14}C-labelled, non-extractable simazine residues was investigated in a standardized plant test. The detailed result of these experiments are published (Kloskowski and Führ, 1983 a and b).

TEST METHODS, RESULTS

The Ap layer of a Parabraunerde (Alfisol) was air dried, sieved and |ring-^{14}C|simazine was applied. The concentration of the added simazine was calculated, taking into consideration the degradation rate, the phytotoxicity to maize and the expected extraction yield. It amounted to 5 to 8 mg simazine/kg dry soil. In order to increase the fertility of the soil 1 g of complete fertilizer was added per kiligramme soil and microorganism activity was initiated by a fresh suspension of garden soil. Incubation took place in desiccators containing 5 kg of soil at 22°C, 65 % of the maximum water capacity of the soil (WK_{max}) in darkness. At the same time 3 kg of simazine-free soil was incubated as a control under identical conditions. Air free of carbon dioxide was passed over the soil. Carbon dioxide from soil respiration and ^{14}C carbon dioxide from simazine degradation were absorbed in 1 N NaOH. Degradation lasted 108 days.

There were no significant differences between the two simazine concentrations (5 or 8 mg/kg soil) with respect to mineralization, the order of magnitude of the extracted residues and the fractions of non-extractable residues in the soil. For this reason, the results from the conversion of 5 mg simazine/kg soil are plotted in most of the following Figures.

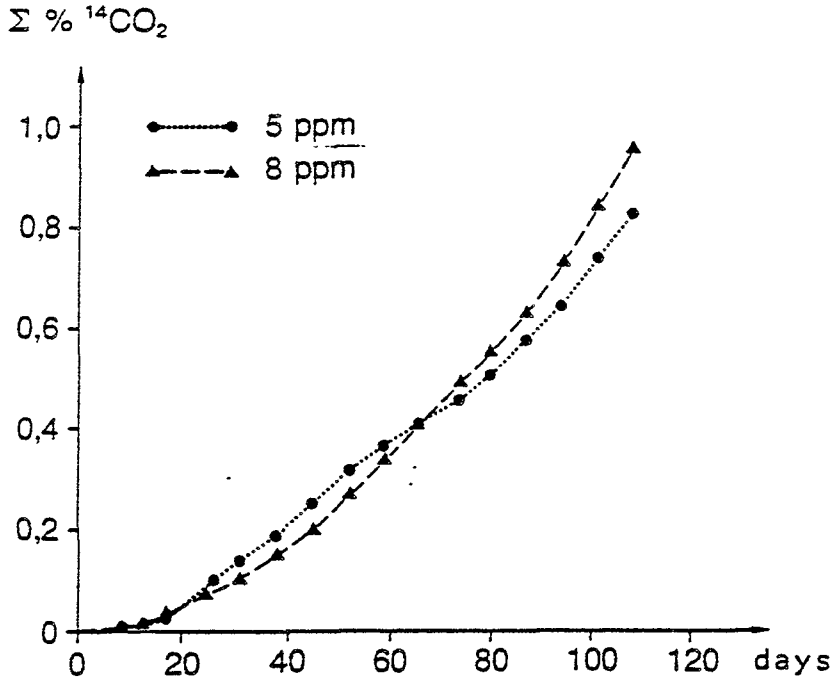

Fig. 2: $^{14}CO_2$ development from a Parabraunerde (Alfisol) during incubation of 108 days with |ring-^{14}C|simazine. Applied radiactivity = 100

After 108 days between 0.8 and 1 % of the applied radioactivity was mineralized to $^{14}CO_2$. Degradation began very slowly. At the end of the experiment increasing mineralization rates were observed. The overall low degradation indicates a high persistence of the s-triazine ring, which was bound more and more strongly to the soil components. Soil specimens for extraction were taken on day 1, 37, 78 and 108. Four different extraction procedures were used on day 78. The extraction mixtures developed by Cheng et al. (1983) of 0.2 M ascorbic acid, 0.2 M citric acid and acetone in the proportions by volume of 1:1:2, gave the highest yield. Extraction with methanol in a Soxhlet extractor or by shaking and a multistage extraction with $CaCl_2$, acetone and methanol were the other methods used.

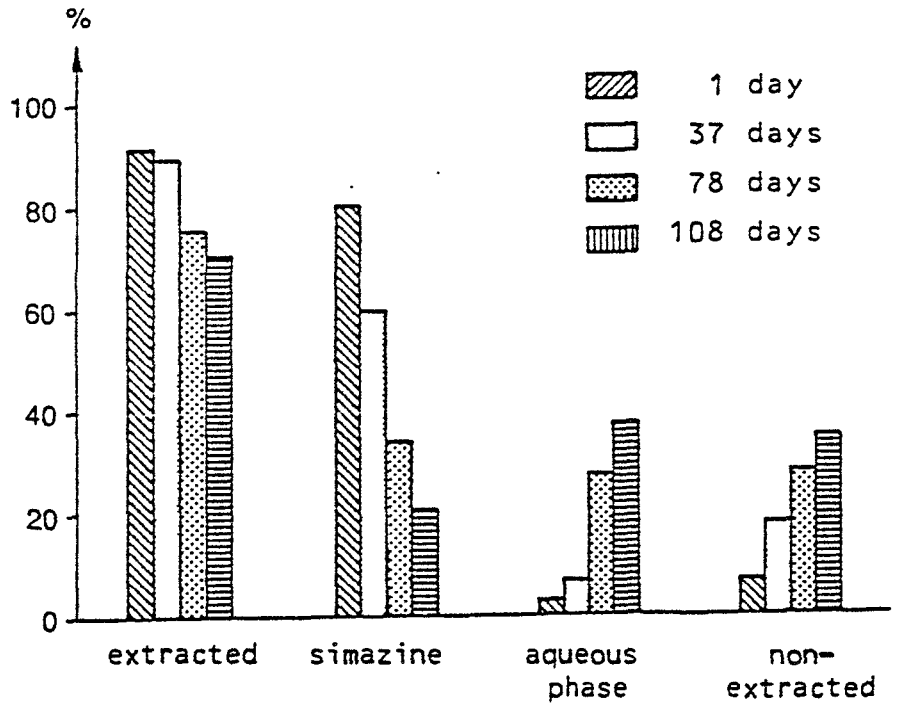

Fig. 3: Extractability of radioactivity after conversion of |ring-^{14}C|simazine under standard conditions in a Parabraunerde (Alfisol) using 0.2 M ascorbic acid, 0.2 M citric acid and acetone (1:1:2). Qualitative and quantitative changes in the course of conversion.
Radioactivity in the soil at the point of extraction= 100 .

About 91 % of the applied radioactivity could still be extracted from the soil one day after application. In the course of time, the extractability decreased from 89 % 37 days after the beginning of conversion to 71 % of the radioactivity still remaining in the soil after 108 days. At the same time the fraction of non-extractable radiocarbon increased from 17 % after 37 days to 34 % after 108 days' conversion.

After one day approximately 80 % of the extracted radioactivity represented unchanged simazine. This simazine fraction was reduced in the extracts from 59 % and 34 % on days 37 and 78 respectively to approximately 20 % on day 108.

The soil extracts were shaken with chloroform and the organic and aqueous phases allowed to be separated. The fraction of radioactivity in the aqueous phase rose from 2.5 % on day 1 and 6.7 % on day 37 to 37 % at the end of the conversion test. This indicates the

formation of polar, water-soluble products, whose characterization was not possible with the aid of thin-layer chromatography.

The total soil volume treated with 8 mg simazine per kg soil was exhaustively extracted on day 108.

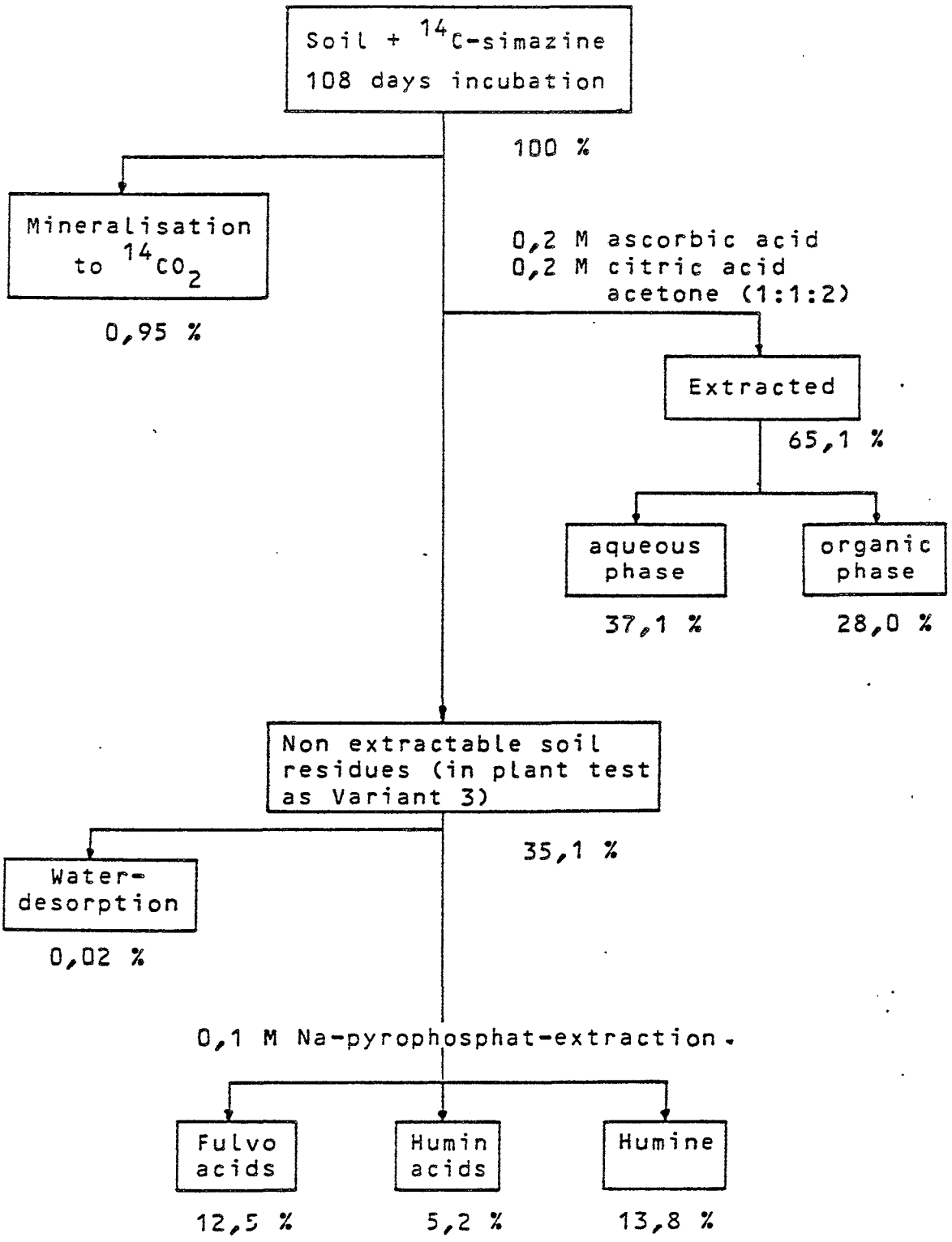

Fig. 4: Production of bound ^{14}C simazine residues for the plant test (extraction plan).

The soil was shaken for four times 4 hours and once for 16 hours with the acid extraction mixtures consisting of ascorbic acid, citric acid and acetone. About 65 % of the radioactivity originally present in the soil could be extracted. 37 % of the extractable radioactivity was present in the aqueous and approx. 28 % in the organic phase of the extract. About 35 % of the radioactivity applied remained bound in the soil. Approximately 18 % of the bound radioactivity was dissolved by an additional extraction of 10 g of the soil residue with 0.1 M sodium pyrophosphate. About 5 % of this could be precipitated with 1 N hydrochloric acid at pH 1 - 2 in the form of humic acid. Approximately 14 % of the radioactivity applied was bound in the humin fraction after 108 days. This soil, as well as non-extracted soil with aged simazine residues, was used for the biotest with maize.

The plant test of the bioavailability of the bound simazine residues was carried out in three test variants, each being repeated three times:

Test variant 1: The active substance |ring-^{14}C|simazine was freshly added to the soil immediately before the plant test and mixed in evenly.

Test variant 2: The soil was incubated for 108 days under standard conditions after the application of |ring-^{14}C| simazine.

Test variant 3: The soil was treated in the same way as in Variant 2, but then subsequently exhaustively extracted with polar and non-polar solvents.

The simazine and simazine equivalent concentration in the plant test was between 2.7 and 3 mg/kg soil. In addition to the test variants, there were 2 control pots each with soil similarly treated to that in the plant test but without simazine.

After initial growth in a nutrient solution, the maize plants at the bifoliate stage were sealed airtight into special pots using Prestik in the openings of the glass lids. The plants grew for 30 days in the isotopic greenhouse at average temperatures of 22°C, auxiliary illumination of approx. 20.000 lux and a day and night

rhythm of 16/8 hours. The test pots were specially designed in order to permit the root atmosphere to be separated from the shoot atmosphere (Mittelstaedt and Führ, 1975).

Air free of carbon dioxide was passed through the soil at a rate approx. 5 l/h. Carbon dioxide from soil respiration and root respiration, as well as $^{14}CO_2$ from the mineralization of |ring-^{14}C| simazine and metabolites, was absorbed in 1 N NaOH. The soil moisture content was adjusted daily to 80 % of the maximum water capacity of the soil in the first week of the test, and then reduced to 65 % WK_{max}. By adding fertilizer and also by inoculating the soils with a suspension of a garden soil before the experiment began, it was hoped to correct losses in fertility in the soil of Variant 3 during the extraction procedure.

After 30 days the shoots were cut off and the roots separated from the soil, freeze dried and pulverized. The soil was air dried. Portion of the plants and soil were incinerated to $^{14}CO_2$ and the radioactivity balance drawn up. The recovery rate was between 98 - 103 %.

The maize plants took up approximately 20 % of the radioactivity from the ^{14}C simazine mixed in immediately before the test began via the roots and translocated the ^{14}C labelled compounds into the maize shoots. Only approximately 10 % of the radioactivity present in the soil from the fraction of aged simazine residues (Variant 2) was taken up and also almost exclusively passed on into the shoots. At 0.08 % only traces of radioactivity became biologically available from the fraction of bound residues (Variant 3). An overall uptake ratio of 260:130:1 resulted.

As a rule, the plants can only take up the organic compounds freely available in the soil solution via the root. There is thus a relatively good agreement between the results of the uptake of radioactivity and the gradient desorbed with water at the end of the test.

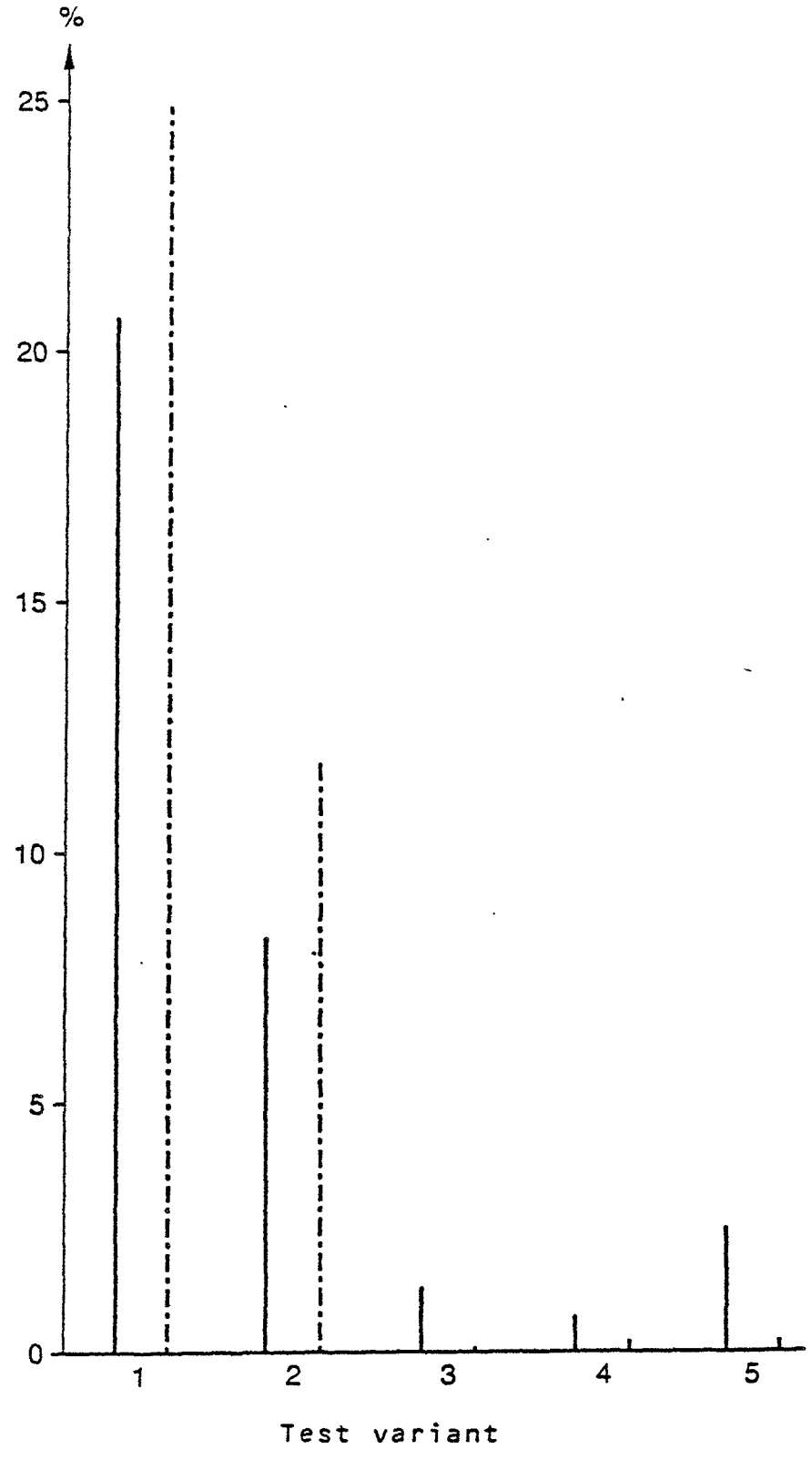

Fig. 5: Desorption of radioactivity with H_2O and uptake of radioactivity by the maize plants.
Radioactivity in the soil after the plant test = 100

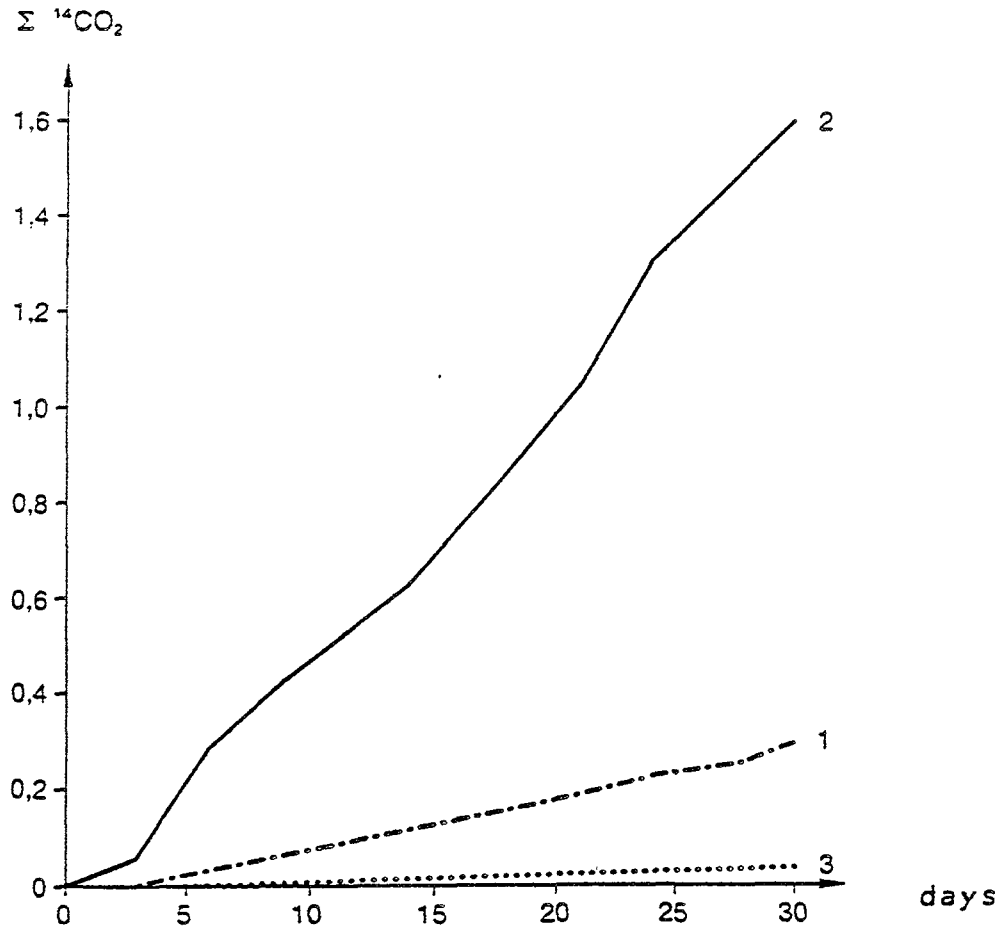

Fig. 6: $^{14}CO_2$ formation in planted soil containing ^{14}C-simazine residues.
Radioactivity in the soil at the beginning of the test = 100

Variant 1: Soil + ^{14}C-simazine applied directly before beginning the test
Variant 2: Soil + ^{14}C-simazine incubated for 108 days
Variant 3: Soil + ^{14}C-simazine incubated for 108 days, exhaustively extracted.

During the 30-day plant test a maximum of 1.6 % of the ring carbon of the simazine molecule was mineralized to $^{14}CO_2$. This value refers to mineralization in the soil of Variant 2, which was previously incubated for 108 days with simazine. 0.3 % of the radioactivity was converted to $^{14}CO_2$ if simazine was added to the soil immediately before the plant test. The non-extractable aged residues remained largely stable with respect to mineralization.

The soils of the three test variants were extracted after the plant test.

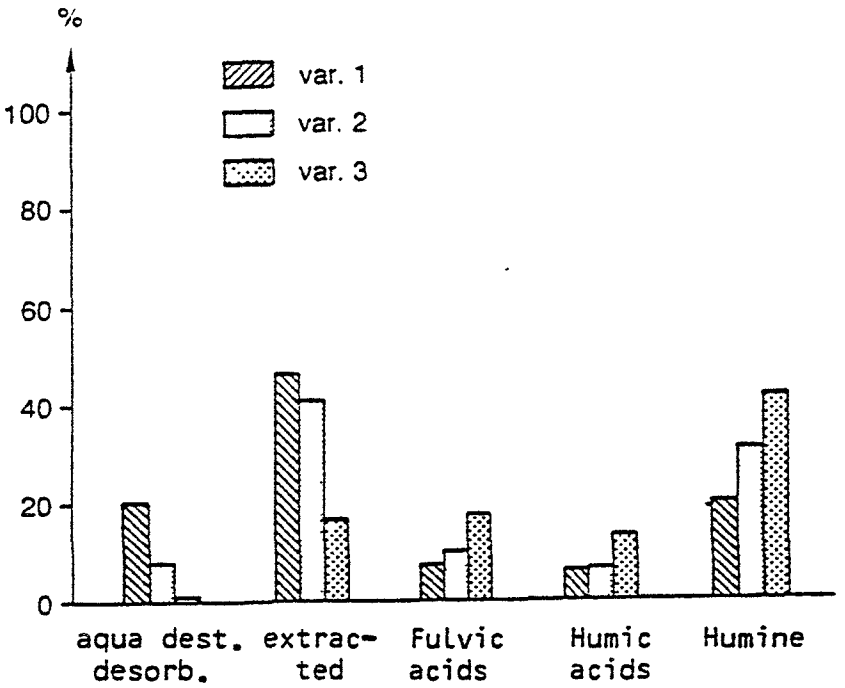

Fig. 7: Distribution of radioactivity in the soil after the conclusion of the plant test with ^{14}C simazine. Radioactivity in the soil = 100

21 % of the radioactivity remaining in the soil could be extracted with distilled water from the soil to which |ring-^{14}C|simazine was added immediately before beginning the test (Variant 1). 8 % of the radioactivity could to be extracted from the soil of Variant 2 (with aged residues). Only 1.3 % of the radioactivity remaining in the soil of Variant 3 was soluble in water. 47 and 41 % of the radioactivity applied could be extracted from the soils of Variants 1 and 2 respectively with 0.2 M ascorbic acid, 0.2 M citric acid and acetone (1:1:2). In contrast, only 17 % could be extracted after the 30-day plant test from the soil of Variant 3 which already had been exhaustively extracted with the solvent mixture before beginning the test.

The remaining soils of the Variant 1, 2 and 3 were then extracted with sodium pyrophosphate and the radioactivity measured in the fractions of fulvic acids and humic acids. The fixing of simazine and its metabolites into the stable carbon fraction of the soil apparently takes place very rapidly. Approximately 20 % of the ring carbon from the simazine molecule was detected in the humin fraction within 30 days. In the soil of Variant 2, in which the

simazine and simazine residues had already been subjected to a degradation process for 108 days before the beginning of the test, this fraction amounts to 31 % after the plant test. This fraction was increased in the case of ring carbon which had already been defined as non-extractable before the test began.

It must be said at this point that the relatively drastic treatment of the soil in Variant 3 before the plant test clearly affected the degradation and conversion processes as well as plant growth uptake of solutes. To check whether the biological activity had been reestablished during the plant test in the extracted soil of Variant 3, an additional experiment was carried out on the degradation of ^{14}C glucose in control treated soil samples.

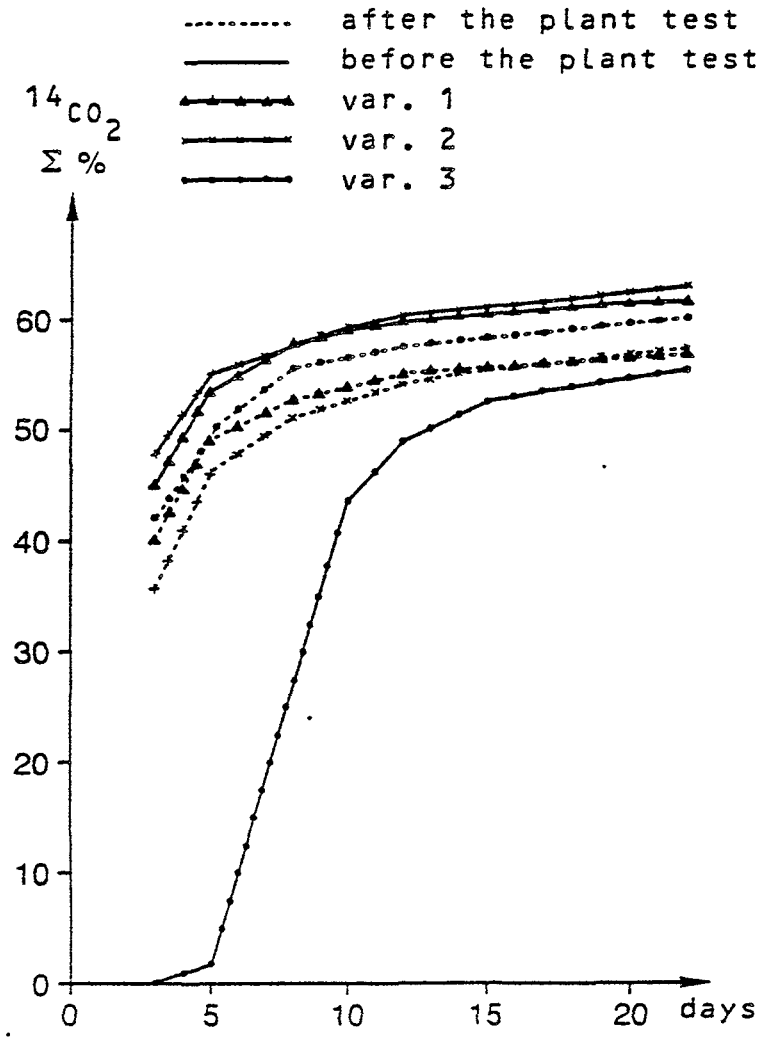

Fig. 8: D|^{14}C-U|glucose degradation in control soils of the three test variants of the plant test before and after plant growth.
Radioactivity applied = 100

With one exception, approximately 50 % of the glucose carbon was mineralized to $^{14}CO_2$ within 5 days. The degradation curves are largely identical with 55 - 60 % mineralization after 22 days.

Mineralization of the glucose carbon in the soil of Variant 3, which had been exhaustively treated with the acid extraction mixture before the plant test, took a different course. Degradation only began after 3 days and took 2 weeks to reach the level of the other control soils. After the plant test, the same soil did not differ from the non-extracted soils with respect to glucose degradation. It can thus be concluded that the development of the biomass was positively influenced during the maize test in the soil of Variant 3.

In an additional test, the soil of Variant 3 was therefore used again in the second plant test under the same conditions as the first plant test as follows:

Variant 4: The soil from Variant 3 was replanted after the plant test.

Variant 5: After the plant test the soil of Variant 3 was mixed with 50 % fresh, untreated soil.

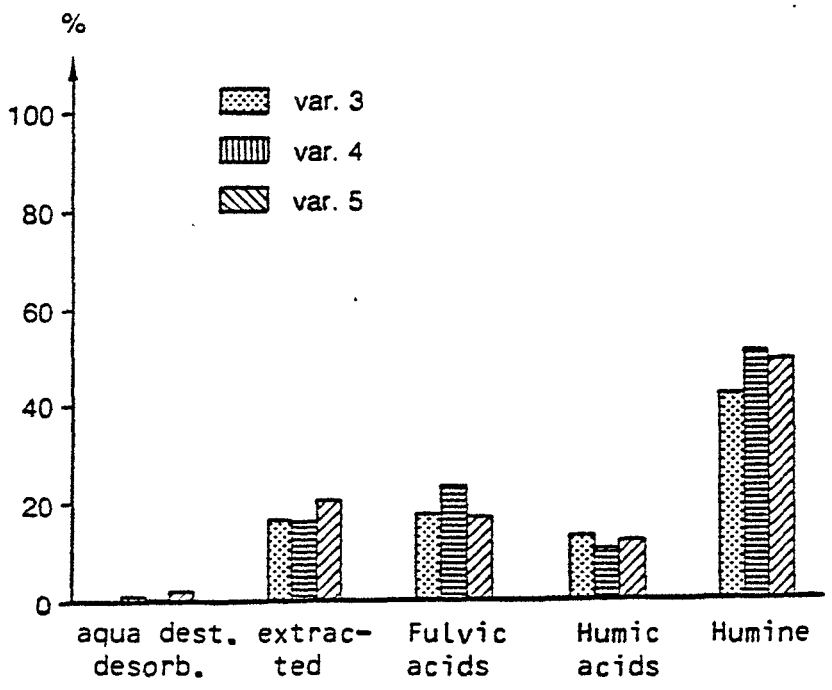

Fig. 9: Distribution of radioactivity in the soil after concluding the plant test (Variants 4 and 5). Comparison with the distribution in the soil of Variant 3.
Radioactivity in the soil after the plant test = 100.

The mineralization and uptake of radioactivity by plants were similar in Variant 4 and Variant 3.

The addition of fresh soil (Variant 3) increased the rate of the degradation and conversion processes, but did not significance affect the distribution of radioactivity in the extracts and fulvic and humic acid fractions.

These results show that simazine is relatively stable in the soil, but nevertheless is already incorporated into the stable and instable carbon stock of the soil within a few weeks. In addition to microbial degradation processes and biochemical reactions, sorption processes are also involved. This leads to a reduction in the biological availability to plants in particular the biological availability of the residue fraction which can no longer be extracted with polar and non-polar solvents, that is to say the bound residue, is drastically reduced.

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FURTHER STUDIES ON THE BOUND PESTICIDE RESIDUES IN SOIL AND PLANTS

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Abstract

Bound residues in soil occur with many pesticides and/or their transformation products (9). Recent reports in the literature clearly point out that the bound residues can be released from the soil and absorbed by plants (2,3,10-12). The data presented in this paper further demonstrate that the various physiological groups of microbes used in this study have the enzymatic and/or biological potential to release bound pesticide residues from soil. The release was not influenced by the nature of the microbial population present, thereby indicating that the process involved cometabolism or that the residues are bound in various matrices, or both.

Our studies also indicate that the in vitro incubation of chicken liver homogenate with plant tissues containing bound ^{14}C residues released and metabolized some of these residues. However, no apparent release of ^{14}C residues was observed when the incubation involved rumen liquor. It appears that in the liver homogenate incubation system, bound 2-chloro dealkylated metabolites in the plant tissues were released and subsequently metabolized to 2-hydroxy analogues. In our earlier studies it was shown that in the metabolism of atrazine by the soluble fraction from chicken liver homogenates, the hydrolysis reaction predominates resulting in the formation of 2-hydroxy analogues which do not undergo further degradation by dealkylation (13).

Only a few studies have been reported on the bioavailability of plant bound residues to animals (14-17). It appears that bound residues in plants

are not available to the animals since, in nearly all the studies reported, elimination of bound ^{14}C residues has been found rapid and via the feces. Apparently, absorption of bound residues from the gastrointestinal tract will depend upon release of these residues from the plant matrix. The released residues will then accumulate in an effective concentration in solution at the site of absorption and subsequently result in permeation through the gastrointestinal barrier (18). This did not occur with the bound residues in plant material in the studies reported earlier (14-17). Our data showing only a very small release of bound ^{14}C residues when incubated with rumen liquor substantiate the earlier findings.

Introduction

In recent years bound (nonextractable) residues of pesticides in soil and plants have been the subject of an extensive investigation in our laboratory. Earlier, we reported a high temperature distillation (HTD) technique for determining and chemically identifying the soil- and plant-bound residues of the s-triazine herbicide prometryn [2-(methylthio)-4,6-bis(isopropylamino) -s-triazine] (1). Application of this technique in our studies revealed that a considerable portion of the bound ^{14}C-residues in the incubated organic soil treated with ^{14}C-ring-labeled prometryn was present in the form of the parent compound (1). The bound ^{14}C-residues were absorbed by plants grown in the soil (2). Mono- and di-N-dealkylated metabolites of prometryn were present in the plant bound ^{14}C-residues and a major portion of bound residues was associated with lignin (2). Bound ^{14}C-residues were also released from the soil when incubated with a liquid inoculum from the untreated field soil (3). The bound residues in the soil were associated with humic fractions (4). It was suggested that, in addition to chemical binding, the pesticides and/or their metabolites are also firmly retained by the

organic fraction (humic materials) by a process that more likely involves adsorption on external surfaces and entrapment in the internal voids of a molecular sieve-type structural arrangement (4).

Results of the foregoing studies were reported in the first FAO/IAEA Research Coordination Meeting held at San José, Costa Rica. We now report the results of our further investigations concerning the release of bound ^{14}C-residues from soil and plants by various physiological groups of microbes and liver enzymes homogenates, respectively.

Materials and Methods

I. Release of soil bound ^{14}C-residues:

Soil. Soil containing bound ^{14}C residues was obtained as described in an earlier study (3). The soil contained 57.4% bound ^{14}C of the total initially applied ^{14}C. More than half (54%) of the total bound residue in soil was in the form of prometryn. The remainder constituted hydroxypropazine (8%), traces of mono-N-dealkylated analogues of prometryn, unidentifiable methanol-soluble material (~18%) and 20% was thermally decomposed to $^{14}CO_2$ during identification procedure involving a high temperature distillation (HTD) technique (1).

Soil Sterilization. Ten gram aliquots of air-dried soil (methanol extracted) containing bound ^{14}C residues were placed into screw capped pyrex test tubes (50 ml capacity). The tubes were closed and then exposed to γ-irradiation to receive a total dose of 2.5 megarads. The control consisted of untreated soil exhaustively extracted with the same solvents as those used for producing bound residues. The control sample was also exposed to γ-irradiation as described above. The sterility of the soil samples was checked by plating the soil on potato dextrose agar and soil extract agar followed by incubation of plates at 20°C for 2 weeks.

Isolation of the Physiological Groups of Microbes. Four different physiological groups of microbes were isolated from the prometryn treated soil (4.48 kg/ha). Isolation was based on their ability to degrade substrate added to agar. Agar containing casein, vanillin, ground cellulose and egg yolk was used for isolating proteolytic, lignolytic, cellulolytic and lipolytic microbes, respectively. For each group, about 40 pure cultures were isolated from randomly chosen colonies on the agar plates. For lignolytic, all colonies on the agar were presumed to be utilizers of the substrate. For the remaining three groups, only those showing zones of degradation around the colonies were counted as positive. Microscopic examination of wet and stained mounts prepared from the selected colonies showed that the celluloytic and lignin-like decomposers were mainly fungi, the proteolytic gram variable cocci and motile short rods, the lipolytic gram positive cocci and long rods. In general these observations agree with those reported elsewhere (5).

Warburg Experiment. A Warburg apparatus was used to evaluate the ability of each physiological group of microbe to release and/or degrade bound residues. Six gram (oven-dry basis), of air-dried sterilized soil containing ^{14}C bound residues was transferred to a 125 ml capacity Warburg vessel and 12 ml of an inoculum was added (soil moisture - 65% of field capacity). The inoculum, which contained a suspension of one of the physiological groups of microbes or a mixture of all four groups, was prepared by suspending about 3 g (wet weight basis) of growth from agar plates in 100 ml sterilized water. The sterilized control soil sample was also placed in Warburg vessel and inoculum added. All systems were duplicated. Rigid aseptic procedures were followed in the transfer of soil and inoculum in the Warburg vessel and the subsequent oxygen uptake measurements. KOH (20%) was used in the centre wells of the vessels to trap $^{14}CO_2$ and the temperature maintained at 20°C. Oxygen uptake was measured daily for a period of 28 days. The endogenous oxygen consumption was determined by adding in two vessels of each soil 12 ml

sterilized water only. The values were subtracted from the oxygen uptake of the systems containing the added microbes. At the end of the experiment, KOH from the centre well was removed and the radioactivity was determined. The soil was removed from the Warburg vessel and a portion of the the soil was processed to determine extractable and bound residues as described earlier (3). The other portion was used to determine the total numbers of each physiological group of microbes by plating suitable dilutions of the mixture on the appropriate agar. Microbial contamination was checked by plating soil from the endogenous systems on potato dextrose agar and soil extract agar. Plates were poured in quadruplicate and incubated for 2 weeks at 20°C.

II. Release of plant bound ^{14}C-residues:

Plant Material. Ten corn plants (Zea maise), 19 days old, were exposed to 5 ppm aqueous solution of ^{14}C-ring-labeled atrazine (0.16 μCi/jar). The plants were grown in Hoagland nutrient solution (300 ml/jar) and were maintained in a growth chamber for 8 days after herbicide treatment. Nutrient solution was added intermittently to the jar to replace loss due to transpiration over the growing period. At the end of the treatment period, each plant was harvested and the shoots and roots were separated. The latter was washed with cold water and the shoots and roots were stored at -20°C until analyzed. Aliquots of the dried (24 h at 30°C) plant tissues were combusted to $^{14}CO_2$ for determination of the total ^{14}C residues.

Generation of Bound ^{14}C Residues in Plant Tissues. Both root and shoot samples were thawed at room temperature and blended at high speed with dried chloroform (1:100 w/v) for 5 min. The mixture was filtered under suction and the sample residue washed with chloroform. The insoluble material from root or shoot tissue was blended at high speed with methanol (1:100 w/v) for 5 min. The mixture was filtered under suction, and the insoluble sample residue was blended again with methanol (1:100 w/v) and finally with water

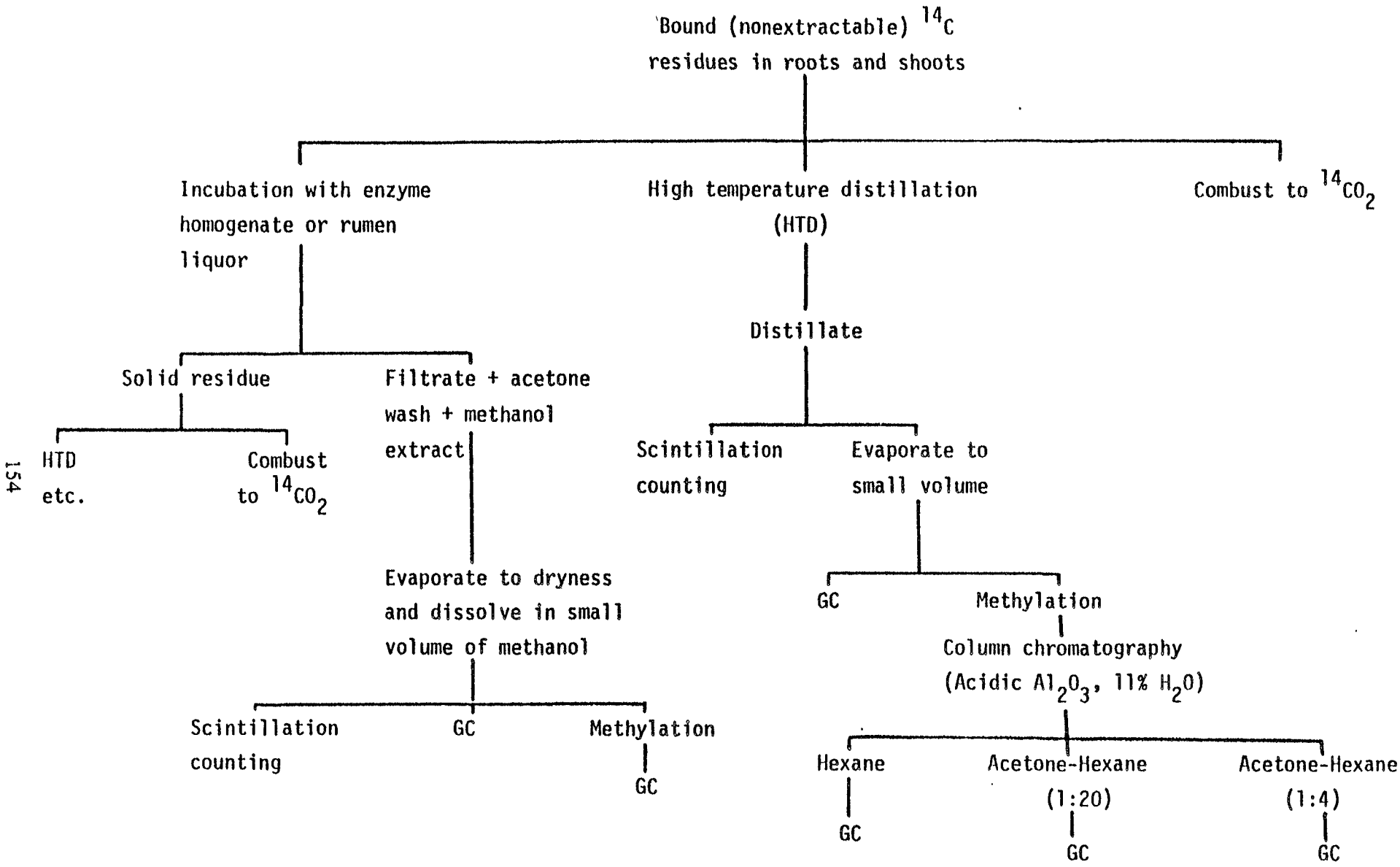

Figure 1. Schematic diagram for the analysis of bound ^{14}C residues in plant tissues and the extractable ^{14}C residues after incubation.

(1:100 w/v). Further blending of the insoluble material with solvents did not result in any extractable ^{14}C. The insoluble shoot or root samples containing only bound ^{14}C residues were dried at 30°C for 24 h and were divided into three parts. One part of shoot or root sample was combusted to $^{14}CO_2$ to determine the total ^{14}C. The other part was used to release bound ^{14}C by the high temperature distillation (HTD) technique (1), subjected to column clean up and finally analyzed by gas chromatography as shown in Figure 1. The third portion was used for incubation studies as described below.

Preparation of Liver Homogenate and Rumen Liquor. A 20% (8 g liver in 40 ml of buffer) homogenate of chicken liver was prepared in 0.134 M phosphate buffer, pH 7.4. The homogenate was centrifuged at 2500 rpm for 10 min at 4°C. The liver-homogenate thus prepared contained mitochondria, microsomes and soluble fraction.

Rumen liquor obtained 3 to 4 h post-feeding from a fistulated cow fed corn silage was strained through four layers of cheesecloth. The filtrate was centrifuged at 1600 rpm for 20 min. The supernatant layer was decanted and used as inoculum media.

In Vitro Incubation. (i) Liver Homogenate. Ground plant material (100 mg) was suspended in 30 ml of freshly prepared liver homogenate in glass-stoppered Erlenmeyer flasks (50 ml) and incubated at 37.5°C for 5 h. Control flask contained inactivated or no liver homogenate. In the latter case the volume was kept constant by adding an appropriate amount of buffer (30 ml). At the end of the experiment the enzymatic activity was destroyed by addition of 30 ml acetone to the flasks. The mixture from each flask was filtered, the reaction flasks were rinsed with acetone (10 X 10 ml), the rinse was again filtered and combined. (ii) Rumen Liquor. Ground plant material (100 mg) was incubated with rumen liquor (50 ml) at 37.5°C for 3 h. The control consisted of inactivated rumen liquor. At the end of the incubation

period, the mixture was diluted to 100 ml with methanol and then centrifuged at 10,000 rpm. The supernatant was passed through filter paper.

Extraction and Determination of ^{14}C Residues. The analytical procedure used for the extraction, isolation and determination of ^{14}C residues after incubation is shown in Figure 1. The insoluble material from root or shoot tissue after incubation and filtration as described above was exhaustively extracted with methanol. The methanol extract was combined with the respective filtrate described above, evaporated to a small volume and processed as shown in Figure 1. The residual extracted roots and shoots containing bound (nonextractable) residues were dried at 30°C for 24 h and were combusted to $^{14}CO_2$ to determine the total ^{14}C.

III. Analytical techniques:

Determination of Radioactivity. Combustion of the dried soil or plant tissues was done in a Packard sample oxidizer, Model 306, to produce $^{14}CO_2$. The radioactivity of aliquots of various extracts or KOH was determined by liquid scintillation counting (1).

Gas Chromatography (GC). The gas chromatograph was a Varian Model 6000 fitted with a thermionic specific detector. The column was a 1.8 m X 0.2 cm i.d. glass tube packed with 3% Carbowax 20 M coated on 100-120 mesh supelcoport. The operating conditions were: column, detector and injector port temperatures were 190, 300 and 220°C, respectively. The nitrogen carrier gas, hydrogen and air flow rates were 20, 4 and 150 mL/min, respectively.

Confirmation. The identity of the compounds was confirmed by comparing the GC retention times with those of authentic samples, cochromatography, and finally by gas chromatography-mass spectrometry. A high resolution mass spectrometer Model VG 2AB-2F connected to a Varian GC Model 3700 was used. The mass spectra were recorded at 70 eV.

Results and Discussion

I. Release of soil bound ^{14}C-residues by microbes:

γ-irradiation effectively sterilized the soil as indicated by absence of microbial growth on agar plates prepared from the treated samples at the beginning of the experiment and those from the endogenous systems at the end of the experiment. Furthermore, the absence of growth in the endogenous systems indicated that contaminating microbes failed to enter any of the vessels. In general, during the incubation period the number of each physiological group increased by about 100 fold (Table 1). This suggests that the release and/or metabolism of bound ^{14}C residues was solely due to the activities of the microbes. In preliminary experiments an aliquot of the γ-irradiated soil was extracted with methanol and radioactivity determined. Analysis of the extracted material revealed negligible amounts of radioactivity indicating that bound residues were not released by γ-irradiation.

Increase in oxygen consumption indicates the utilization of the organic matter as a substrate by the microbes. Figure 2 shows that cellulolytic microbes were most active in the utilizaton of organic matter followed by the mixed population which utilized about 80% of that shown by cellulolytic groups. However, the proteolytic, lipolytic and lignolytic microbes utilized about half the amount of that used by the cellulolytic group. The oxygen uptake of the inoculated soil containing ^{14}C bound residues was nearly identical to that for control soil for each of the physiological groups.

The different microbes were able to release (extractable ^{14}C + $^{14}CO_2$) part of the ^{14}C bound residues from inoculated soil to about the same extent (Table 2). Mixed population of microbes released slightly higher and proteolytic slightly lower amounts of ^{14}C bound residues than the remaining three physiological groups. The results clearly demonstrated that the microbes used in this study can release part of the bound ^{14}C residues

in soil. However, no one physiological group of microbes was considerably more active than the other in releasing the bound ^{14}C thereby indicating that these microbes were not strictly substrate specific under the experimental conditions described. Furthermore, it was not possible to determine the likely competitive ability of these microbes in releasing the bound ^{14}C residues.

Analysis of the extractable residues from the γ-irradiated soil incubated with inoculum of various physiological groups indicated the presence of prometryn (0.19 - 0.25 ppm), hydroxypropazine (0.80 - 0.92 ppm), mono-N-dealkylated hydroxy-propazine (0.1 ppm) and traces of mono-N-dealkylated prometryn. Thus, in terms of prometryn equivalent, the total extractable material identified amounted to about 24 - 26% of the initially bound prometryn present before incubation. The bound (nonextractable) residues from the soil after incubation were released by the HTD technique and the distillates were subsequently analyzed (1). The results indicated that prometryn (1.39 - 1.69 ppm), hydroxypropazine (0.22 - 0.25 ppm), and mono-N-dealkylated prometryn (0.36 - 0.54 ppm) were still present in the form of bound residues. It was observed that the bound (nonextractable) prometryn after incubation still amounted to about 39 - 44% of the initially bound herbicide. Release of bound residues by the HTD technique also resulted in thermal decomposition to $^{14}CO_2$ (~20%) during distillation (1). Furthermore, considerable variation in the recoveries of the hydroxy analogues have been observed due to the poor efficiency of methylation (6). Therefore, the residue data reported here should be regarded as being qualitative only. The results show that the bound residues released during incubation were metabolized by the microbes via hydrolysis accompanied by partial N-dealkylation. This is evident by the presence of extractable residues of metabolites containing both 2-hydroxy and 2-methylthio metabolites. It is also possible that some of the products formed during the incubation period

Table 1. Number of physiological groups per g of oven-dry soil

Physiological groups	Number of physiological groups	
	Beginning of experiment	End of experiment
Lipolytic	43×10^6	52×10^8
Proteolytic	37×10^6	23×10^8
Cellulolytic	16×10^6	19×10^8
Lignolytic	40×10^6	9×10^8
Mixed	34×10^6	26×10^8

Table 2. Distribution of ^{14}C from inoculated soil containing bound ^{14}C-residues after 28 days incubation in a Warburg apparatus.

Physiological group	^{14}C (% of the total bound ^{14}C before incubation)		
	$^{14}CO_2$	Extractable	Bound
Lipolytic	3.0	25.2	71.7
Proteolytic	2.0	23.5	73.9
Cellulolytic	1.4	27.1	70.7
Lignolytic	1.7	26.8	71.4
Mixed	2.9	27.0	70.0

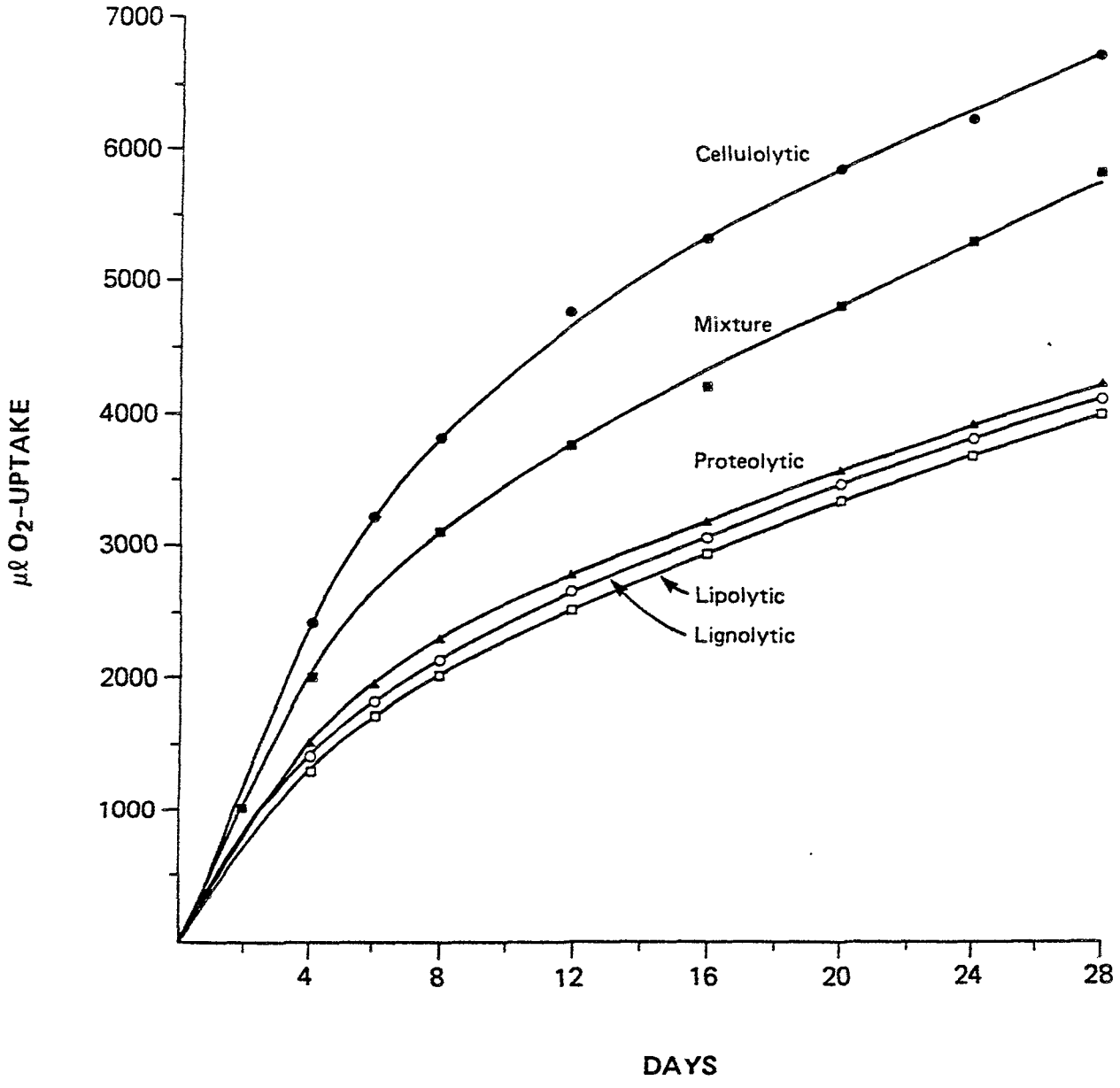

Figure 2. Oxygen uptake of soil containing bound ^{14}C residues, incubated with four different physiological groups of microorganisms and their mixture.

may have undergone a slow binding process to become a part of bound portion of the residues in soil (1,9).

Evaluation of $^{14}CO_2$ from microbial systems indicates mineralization or ring cleavage of the released and/or bound residues in the incubated soil. In other studies, the evaluation of $^{14}CO_2$ from ring-labeled s-triazine has been taken as evidence for ring cleavage and there are many varied reports of the extent to which this occurs in soils (7). There appears to be a general

agreement that evolution of $^{14}CO_2$ from ring-labeled 2-hydroxy analogues of s-triazine is greater than from analogues containing 2-chloro or 2-methylthio moieties (7,8). Whether or not the ^{14}C in the evolved $^{14}CO_2$ was actually derived from the extractable hydroxylated analogues during incubation, the parent compound, the bound residues, or all is not known. Furthermore, no explanation can be offered at this time for the observed variation in the capacity of microbial systems to liberate $^{14}CO_2$ in the incubation experiment (Table 2).

II. Release of plant bound ^{14}C-residues:

The corn plants contained 51.9% of the ^{14}C applied in the hydroponic solution. Table 3 shows the distribution of ^{14}C residues in corn plants. The roots of corn plants contained 49.0% of the total plant ^{14}C whereas the remaining ^{14}C (51.0%) was present in the shoots. Amounts of total extractable ^{14}C residues were larger in roots, but were smaller in shoots accounting for 33.7 and 28.1%, respectively of the total ^{14}C residues (Table 3). The chloroform extracts (less polar residues) contained a lower proportion of ^{14}C than the methanol-water extracts (more polar residues).

Bound ^{14}C remaining in the solid material from roots and shoots after exhaustive solvent extraction amounted to 15.3 and 22.9%, respectively, of the total ^{14}C residues (Table 3). GC analysis of the HTD distillates (1) indicated the presence of mono-N-dealkylated and traces of di-N-dealkylated compounds, namely 2-chloro-4-amino-6-isopropylamino-s-trazine (deethyl-atrazine), 2-chloro-4-ethylamino-6-amino-s-trazine (deisopropyl-atrazine), and traces of 2-chloro-4,6-diamino-s-trazine (ammeline). Trace quantities of hydroxyatrazine and 2-hydroxy-4-amino-6-isopropylamino-s-trazine (deethylhydryoxyatrazine) were also detected in the HTD distillates of plant tissues. Under the experimental conditions described we were able to confirm the identity of about 80-85% bound ^{14}C residues in the plant tissue.

Table 3. Distribution of ^{14}C residues in corn plants

Plant tissue	Bound ^{14}C (%)	Extractable ^{14}C (%)		
		Chloroform	Methanol/water	Total
Root	15.3	5.9	27.8	33.7
Shoot	22.9	8.9	19.2	28.1

The solvents used and the extraction method employed in this study appeared to effectively remove all the extractable ^{14}C residues from plant tissues. However, incubation of plant tissues containing bound ^{14}C residues with buffer (pH 7.4) alone resulted in a further release of some ^{14}C residues (Table 4). Incubation or extraction with aqueous buffers is not commonly employed in residue analysis methodology. In our study only the nonextractable ^{14}C residues in plant tissues after their exhaustive solvent extraction as described earlier were designated as bound residues. Thus, it appears that the ^{14}C residues released in buffer were less tightly bound than those remaining in the plant tissue matrix. Analysis of the material released by buffer from the root or shoot samples showed the presence of the 2-hydroxy analogue of deethylatrazine and traces of hydroxyatrazine.

In vitro incubation of other extracted plant tissues indicated that liver homogenate released bound ^{14}C residues (Table 4). Thus, by the end of the 5 h incubation period with liver homogenate, 36.6 and 17.5% of the bound ^{14}C residues were released from roots and shoots, respectively. The corresponding release of ^{14}C residues with buffer alone or inactivated liver homogenate was considerably less (Table 4). These observations are in agreement with an earlier study indicating the ineffectiveness of inactivated liver homogenate on the degradation of some insecticide (19). The amounts of bound ^{14}C in roots and shoots after incubation with liver homogenate were still higher

Table 4. *In Vitro* Release of Bound ^{14}C Residues from Plant Tissue after Incubation with Liver Homogenate and Rumen Liquor Homogenate and Rumen Liquor[a]

Incubation	^{14}C released, % of the total	
	shoot	root
Buffer	2.6	13.7
Buffer + Liver homogenate	17.5	36.6
Buffer + Deactivated liver homogenate	3.4	11.1
Rumen liquor	4.4	11.8
Deactivated rumen liquor	5.9	10.7

[a]Data are means from duplicate incubation.

amounting to about 63.4 and 82.5%, respectively of the originally bound ^{14}C residues in the plant tissue. The ineffectiveness of rumen liquor in releasing bound ^{14}C residues from the plant tissue was apparent in that only a relatively small but similar amounts of extractable ^{14}C residues were present at the termination of incubation with both fresh and inactivated samples (Table 4). Thus, the release of bound ^{14}C residues appears to be mainly due to the rumen liquor media (ph 6.5-6.9) rather than its action on cellulose containing ^{14}C residues. Cellulose is known to be hydrolyzed or digested by the microbial activity of rumen fluid (20). Thus, it appears that no measureable ^{14}C residues were present in the cellulose fraction of the corn plant. In our studies incubation experiments were carried out for a relatively short duration as we were mianly concerned with the initially released aterials rathern than secondary products.

Analysis of the extractable ^{14}C residues from the incubation mixture of liver homogenate with the plant tissues revealed the presence of only 2-OH

analogues of deethylated and deisopropylated atrazine. Furthermore, the root incubation mixture with liver also showed the presence of an additional di-N-dealkylated hydroxy metabolites. Several other unknown peaks were also observed in the extracts of the incubation mixtures of both liver homogenate and rumen liquor but were not identified because of their very low individual concentrations and the unavailability of their reference standards. In our preliminary experiments low recoveries of hydroxy metabolites of atrazine were obtained due to poor efficiency of methylation (50-60%). Thus, no attempt was made to quantitate the residues identified in the extract of the incubation mixtures.

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2. INTERIM REPORT ON THE COMMON EXPERIMENT: MODEL PROTOCOL FOR DETERMINATION OF BOUND RESIDUES IN SOIL

At the 1st Research Coordination Meeting in 1981, a model protocol was developed for the determination of bound residues in soil. Among the essential elements of this protocol were:

a) Soxhlet extraction of air-dry soil with methanol for 24 hours;

b) determination of initial radioactivity in soil by combustion analysis;

c) determination of radioactivity in the soil following methanol extraction (i.e., yielding bound residue (BR) content);

d) determination of radioactivity in the methanol extract (i.e., yielding extractable residues);

e) measurement of the initial soil moisture content; and

f) use of triplicate samples in each analysis.

The purpose of this protocol was to facilitate the coordinated testing by all participants of two soils that contained BR. The objectives of the test were

a) to determine the variability that occurs in such a standard procedure, and

b) to use the protocol as a basis on which to compare alternative procedures for pesticide extraction.

The two soils were supplied by Dr. Klein (Fed. Republic of Germany) in July 1982. That soil designated "A" had been treated in 1976 with (^{14}C)allyl alcohol. The second soil, "H", was treated in 1975 with (^{14}C)hexachlorobenzene. Both treatments were from lysimeters and had been exposed to natural weathering.

At the 2nd Research Coordination Meeting, the common experiment had been completed by nine participants. Analyses are underway in four additional laboratories, and the final results and complete statistical interpretation will be made at the time of the 3rd Meeting.

Table 1 shows the summary averages (± standard deviation), for all current participants, for Soil A. It was immediately apparent that analyses of the initial radioactivity gave a bimodal distribution: four laboratories found ca. 500 dpm/g whereas the other five laboratories reported ca. 900 - 1100 dpm/g. Listing of results is therefore by increasing initial radioactivity. This grouping is generally applicable to the apparent proportions of bound and extractable residues as well, since the "low residue A" gave ca. 85 % bound whereas the "high residue A" gave ca. 98 % bound. The entire lot of Soil A had been mixed at one time and subsampled for distribution, so the reason for this difference is not apparent. Participants have been asked to send subsamples of Soil A to one location (USA/Wheeler) so that re-analysis can be done without the complication of interlaboratory differences.

No grouping of Soil H (Table 2) was apparent, indicating that the sample was presumably homogeneous. The coefficients of variation in the overall average initial, extractable, and bound radioactivity were, respectively, 12, 26 and 34 %. Error increased in the expected direction. The ^{14}C from hexachlorobenzene remained largely extractable (ca. 85 %), in contrast to allyl alcohol-derived ^{14}C.

Four laboratories conducted additional modifications to the common protocol, including the type of Soxhlet thimble (no effect), combustion method (no effect), and premoistening the soil. The last treatment improved extraction of ^{14}C from Soil A in one study and had no effect on results from another lab; both laboratories found that premoistening the soil did not increase extraction of ^{14}C from Soil H. Other organic solvents were tested, but none were as effective as methanol. Similarly, both batch and steam distillation methods were substantially less efficient than the Soxhlet procedure in extracting ^{14}C.

In summary, the common protocol for bound residue determination worked well. The Committee agreed that a 24-hour Soxhlet extraction with methanol was an efficient general method, while recognizing that other solvents may be more appropriate for other residues. The importance of good soil sampling procedures was stressed, as was the necessity for use of ^{14}C-standards and appropriate soil blanks to correctly adjust for background, quenching, and $^{14}CO_2$ trapping efficiency.

Table 1. Collaborative extraction of Soil A

Laboratory		Initial	Radioactivity, dpm/g			Residue % of initial	
Location	Investigator	moisture content, %	Initial	Extractable	Bound	Extractable	Bound
W. Germany	Scheunert	5.0 ±0[1]	480± 6[1]	45± 4	454±84	9.6±1.3	86.0±15.7
Pakistan	Hussain	1.06±0.34	483±17	44± 5	412±34	9.2±0.8	85.6± 9.7
Egypt	Mostafa	1.74±0.05	490±50	155±11	315±25	31.4±2.7	64.3± 2.4
Canada	Khan	2.9 ±0.7	533±41	17± 4	515±22	3.3±0.8	97.1± 7.4
		2.7 ±1.7	497±25	65±61	424±84	13.4±12.4	83.3±13.7
USA	Wheeler	1.87±0.06	885± 71	48± 4	827± 58	5.4± 0.9	93.6± 3.0
India	Raghu	1.39±0.01	936±129	56± 8	1011±205	6.1± 1.8	109.7±26.3
Great Britain	Hance	2.17±0.03	1072± 21	68±15	1050± 8	6.4± 1.3	97.9± 1.3
USA	Helling	2.07±0.06	1094± 41	65± 3	1045± 42	6.0± 0.1	95.5± 2.9
Costa Rica	Carazo	1.80±0.01	1147± 64	76± 5	1105± 43	6.6± 0.8	96.4± 2.1
		1.86±0.30	1027±111	63±11	1008±106	6.1± 0.5	98.6± 6.4[2]

[1] One replicate, with 28 % initial moisture content and 796 dpm/g initial residue, was omitted from calculations

[2] Average BR (± standard deviation) if value from India is deleted is 95.6 ± 1.8 %

Table 2. Collaborative extraction of Soil H

Laboratory		Initial	Radioactivity, dpm/g			Residue	
Location	Investigator	moisture content, %	Initial	Extractable	Bound	% of initial Extractable	% of initial Bound
W. Germany	Scheunert	2.0 ±0.3[1]	5168± 416	3849± 58	704± 95	75.1± 5.0	13.6±0.8
Pakistan	Hussain	1.32±0.30	3925± 97	3335±184	659± 61	85.0± 5.8	16.8±2.0
Egypt	Mostafa	1.89±0.08	3960± 59	3043±149	925±110	76.9± 2.9	23.4±4.9
Canada	Khan	12.6 ±0.3	4392± 127	4144± 5	207± 7	94.4± 2.3	4.7±0.0
USA	Wheeler	1.71±0.06	4316± 216	4012± 23	659± 11	93.1± 5.3	15.3±1.0
India	Raghu	2.02±0.26	5173±1263	3529±228	820±188	71.1±18.3	16.8±6.6
Great Britain	Hance	2.22±0.05	4362± 27	4072±228	554± 17	93.3± 4.3	12.7±0.4
USA	Helling	2.04±0.09	5531± 60	4632± 69	591± 30	83.7± 1.7	10.7±0.4
Costa Rica	Carazo	2.00±0.04	4650± 287	3701±106	969± 49	79.7± 2.9	20.9±1.1
		1.90±0.27[2]	4609± 566	3813±475	676±227	83.6± 8.6	15.0±5.5

[1] One replicate, with 15.8 % initial moisture content, was omitted from the moisture average

[2] Water content of the Canadian samples was excluded from the overall average

3. REPORT

3.1. INTRODUCTION

Through their Joint Division, FAO and IAEA have coordinated a wide range of isotopic tracer-aided studies of trace contaminant problems with particular emphasis given to many problems in developing countries. Being scientifically and problem-oriented, these studies have assisted in defining problem areas and in promoting national research programmes. Isotopic tracer techniques are powerful and often unique tools for the study and control of the problems pertinent to the use of pesticide chemicals.

This programme is primarily concerned with bound pesticide residues in soil, plants and food. Chemical analysis of organic residues almost invariably involves solvent extraction and clean-up. Part of the total residue may be "bound", chemically or physically, and will not be recovered by solvent extraction. Although such bound residues may be neither detected nor determined by chemical analysis, they can be of toxicological significance. Alternatively, being undetected they may be wrongly assigned to some other disappearance factor when trying to account for the total residues. Radiotracer techniques, therefore, provide a unique, appropriate and convenient procedure for the determination of total residues, particularly ^{14}C-, ^{3}H- or ^{35}S-labelled substrates. This constitutes a significant step in the better exploitation of nuclear techniques for the protection of man and his environment.

The first research coordination meeting of this programme was held in San José, Costa Rica, 30 November to 9 December 1981 and led to specific recommendations which called for developing reference methodology to quantify non-extractable pesticide residues, studying the bioavailability of bound pesticide residues and demonstrating their possible biological effects.

3.2. MEETING OBJECTIVES

3.2.1 To review and discuss the progress of the research programme;

3.2.2 To review and discuss the data generated from the common experiment which has been conducted by the programme participants;

3.2.3 To discuss problems pertinent to methodologies and labelled pesticides;

3.2.4 To prepare a progress report of the scientific data and information obtained since the last report and to make recommendations with particular reference to future needs and priorities.

3.3 HIGHLIGHTS

With ^{14}C-lindane it was demonstrated that ^{14}C-bound residues were formed in unflooded and flooded rice fields. Addition of green manure amendment resulted in lower amounts of bound residues compared to unamended soil. When applied to a clay loam soil, ^{14}C-malathion rapidly decomposed to $^{14}CO_2$ with a loss of 56% after 12 days. Bound residues amounted to 38% of the applied radioactivity. Preliminary evidence was presented indicating that bound residues were formed in a rice ecosystem on application of ^{14}C-isoprocarb.

When vinyl-^{14}C-chlorfenvinphos was applied to soils, bound ^{14}C-residues increased with time to 13.7% of the applied dose after 114 days of incubation. Treatment of soil with ring-^{14}C-chlorfenvinphos resulted in higher amounts of bound ^{14}C-residues than those determined with the vinyl label. Experiments with sterile soil suggested that the binding was related to the activity of soil microorganisms. Binding of ^{14}C-chlorfenvinphos was also demonstrated in leaves and stems of rape and the radioactivity was associated with lignin and other materials.

When ^{14}C-paraquat was applied to soil in the field, about 26% of the radioactivity disappeared after 15 months, whereas no loss of radioactivity occurred during one year in the laboratory. Two possible explanations for discrepancy are (1) there was no photolytic decomposition as in the field or (2) the preparation of the soil for the laboratory study upset the microbial ecology of the soil to the detriment of organisms that can degrade paraquat.

The uptake of ^{14}C-endosulfan was demonstrated in tomatoes. Similarly, it was shown that ^{14}C-methomyl was taken up by bean plants. At this stage, the formation of bound residues has not been demonstrated. Studies with ^{14}C-methamidophos demonstrated the formation of bound residues in soil as well as in lettuce and tomatoes.

Radishes treated with ^{14}C-labelled dieldrin, permethrin and carbofuran were shown to contain increasing levels of bound ^{14}C-activity with time. The high temperature distillation technique (HTD) was used to demonstrate the presence of dieldrin, trans-permethrin and two carbofuran metabolites (3-hydroxy and 3-keto carbofuran) in solvent extracted tissues.

The formation of bound residue in soil and plants was investigated with ^{14}C-labelled benzene and chlorinated analogs. The rate of formation of bound residue decreased with an increase in the number of chlorine atoms in the molecule and also paralleled a decrease in resistance to chemical and biological degradation.

The formation of bound residues of ^{14}C-simazine was investigated in soil. Of the non-extractable ^{14}C-residues in soil, only trace amounts of the radioactivity became biologically available to corn grown in the soil for 30 days.

The fate of ^{14}C-ring labelled methyl parathion was monitored during a 49-day period in a silt loam soil. 54% of the radioactivity was present in the soil of which 87% was in the "bound" form. When soils were treated with inorganic and organic amendments and further incubated for 10 weeks, mineralization in extractable and bound residues was observed.

Studies demonstrated that various physiological groups and microbes had the enzymatic/biological potential to release bound pesticide residues from soil. Incubation of chicken-liver homogenate with plant tissues containing bound ^{14}C-residues also resulted in some release and metabolism of the residues. Rumen fluid had no capacity to release ^{14}C- residues.

3.4 ONGOING AND FUTURE RESEARCH

Evidence is currently lacking to indicate whether bound pesticide residues of widely used chemicals constitute a hazard to human health or pose any threat to the quality of the environment. There is much to be exploited on the release, bioavailability and toxicology of bound pesticide residues, and work should continue to generate more information in these areas. Under the FAO/IAEA coordinated research programme, current and future research includes:

3.4.1 Soil-bound residues in the field and in the laboratory of MCPA, linuron, simazine, and tri-allate (United Kingdom);

3.4.2 Soil-bound residues of phenolic and aniline model compounds will be studied in the mineral soil fraction and the humic material as to plant availability, biodegradability by soil microorganisms, and photodegradability (Germany, F.R.);

3.4.3 Studies will continue on soil-bound methamidophos residues and their availability to lettuce and tomato and will be initiated on methomyl or carbofuran binding to soil and plants (Costa Rica);

3.4.4 Investigations on bound residue formation of isoprocarb, carbofuran, carbosulfan, and BPMC in rice paddy ecosystem (soil, rice plant), their bioavailability and possible toxicological significance (Philippines);

3.4.5 Bioavailability of soil-bound ^{14}C-labelled methomyl residues to carrot roots and feeding the extracted carrot roots to mice to demonstrate possible bioavailability and biological effects (Egypt);

3.4.6 Studies on bioavailability of soil-bound chlorfenvinphos residues, their distribution in soil fractions and identification. Winter rape stems containing bound residues will be fed to rodents for possible biological effects (Poland);

3.4.7 Binding of ^{14}C-lindane to soil, bioavailability of bound ^{14}C-residues to rice plants and legumes, and bound residue - microflora interactions (India);

3.4.8 Work will continue on dieldrin, permethrin and carbofuran binding to radish plants in an effort to determine the identity of the bound chemical species, the chemical character of the bound material and its bioavailability (USA);

3.4.9 Completion of investigations on the effect of amendments on ^{14}C-methyl parathion bound residue mineralization with emphasis on the mechanism by which certain treatments accelerated loss of $^{14}CO_2$, and the identification of the chemical species present in the soil extracts (USA);

3.4.10 Bioavailability of soil-bound malathion residues to plants and to microbes and identification of extractable and bound residues (Pakistan);

3.4.11 Investigation of possible binding of endosulfan to tomato fruit and to soils (Sudan);

3.4.12 Bound ^{14}C-deltamethrin residues in soils, plants and animal tissues; bound ^{14}C-dyfonate residues in soil and in onion plants (Canada).

4. RECOMMENDATIONS

In the light of the original objectives and earlier recommendations, the meeting noted with satisfaction that substantial progress has been made. In addition, the following recommendations were addressed to the Joint FAO/IAEA Secretariat:

4.1. To continue to generate data on bound pesticide residues relevant to the individual projects discussed under future work;

4.2. To complete research on the common experiments;

4.3. To encourage participants to investigate common chemicals; e.g. interest to conduct studies with ^{14}C-carbofuran has been expressed by scientists from Costa Rica, Philippines, Egypt, Pakistan, Sudan, India and Poland;

4.4. To encourage further collaboration among programme participants.

It was the consensus of opinion that emphasis should be placed on studies dealing with

4.5. the bioavailability of bound pesticide residues particularly in mammals;

4.6. possible biological effects of bound pesticide residues;

4.7. the identification of the chemical species of bound residues, if relevant to responses under 4.5. and 4.6.

5. CONCLUSIONS

5.1. Data generated under the common experiment were satisfactory, indicating that the proposed experimental model protocol is highly suitable for studies involving bound pesticide residues.

5.2. The bioavailability of bound pesticide residues was demonstrated in corn and microorganisms.

5.3. No biological effects were demonstrated with bound residues (i.e. no phytotoxicity).

5.4. The high temperature distillation technique (HTD) has proven to be a useful tool in the identification of bound pesticide residues.

5.5. With reference to the original objectives and earlier recommendations, the programme has achieved a number of its goals.

5.6. The close contact established between the FAO/IAEA Secretariat and research workers in various countries through the Agency's research contract programme represents a significant contribution to country development, i.e. to assist scientists to identify and study their own problems under their local conditions, to use nuclear and related techniques effectively and to maintain the closest possible contact with their counterparts of the more advanced countries.

6. LOCATION AND DATE OF THIRD RCM

It has been proposed to hold the third meeting at the
Chemistry and Biology Research Institute
Research Branch, Agriculture Canada
Ottawa, Canada
or at the
National Crop Protection Center
University of the Philippines at Los Banos
College, Laguna, Philippines.

Date: June 1985.

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