BOUND RESIDUES OF ^{14}C-CARBOFURAN IN SOIL

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Abstract

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Mineralization of ^{14}C-carbofuran (2,3 dihydro-2,2-dimethyl-7-benzofuranyl-methylcarbamate) to $^{14}CO_2$ as well as the formation of extractable and bound ^{14}C-residues in clay loam soil were investigated under laboratory conditions. The ^{14}C-carbofuran rapidly mineralized to $^{14}CO_2$ and, after 20 days of incubation, 35.6% of the applied ^{14}C was lost as $^{14}CO_2$. The steady decrease of extractable ^{14}C-residues was accompanied by a corresponding increase of bound ^{14}C-residues over a 20-day incubation period. At the end of the experiment, the extractable and bound ^{14}C-residues amounted to 7% and 59%, respectively, of applied radiocarbon. The soil containing bound ^{14}C-residues was fractionated into humic substances. The humic acid, fulvic acid and humin fractions contained 22.39%, 26.04% and 17.56%, respectively, of the applied radiocarbon. The amount of ^{14}C in microbial biomass was 15%.

1. INTRODUCTION

It is now recognized that some chemicals including pesticides can be bound to soil and are unextractable by normal extraction techniques. In view of the growing concern over environmental contamination, it has become extremely important to know about the nature of bound pesticide residues. Soil studies involving tracer-aided techniques can yield information regarding the nature of extractable and bound residues. Numerous investigations have been carried out on bound pesticide residues in soil and plants [1–17].

Soil-bound residues, ranging from 15 to 57%, have been reported for methylcarbamates, a class of compounds with insecticidal and nematocidal properties [18, 19]. While ample information is available in the literature on plant-bound and insect-bound residues of carbofuran [20–22], little is known about its soil-bound residues [23] and the potential uptake by microbes and plants.

This paper reports laboratory work on the bound residues of ^{14}C-carbofuran in soil.

2. MATERIALS AND METHODS

2.1. Chemicals

^{14}C-carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl-[ring-3-^{14}C]-methylcarbamate, specific activity 12.61 mCi/mmol)[1] was supplied by the International Atomic Energy Agency. Radiopurity was better than 99% as checked by radiochromatography. Analytical grade carbofuran was obtained from Bayer AG, Leverkusen, Federal Republic of Germany. All other chemicals used were of analytical grade and all solvents were reagent grade and freshly distilled before use.

2.2. Experimental

The duplicate moist soil samples used (50 g on oven-dry weight basis) had the following physico-chemical characteristics:

Source: NIAB fields
pH (saturated paste): 7.9
Saturation percentage: 32.8%
Organic carbon: 1.8%
Nitrogen: 0.22%
Clay: 18%
Silt: 28%
Sand: 54%
Texture: clay loam.

These samples were placed in Erlenmeyer flasks (250 mL). Acetone (50 μL), containing 17.65 μg of ^{14}C-labelled carbofuran (1 μCi) and 50.55 μg of unlabelled carbofuran, was added to give an insecticide concentration of 1.364 mg per kilogram of soil. 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 $^{14}CO_2$ evolved from soil. The incubation temperature was 30°C and the incubation period was 20 days. On alternate days, distilled water was added to the soil in order to maintain the moisture content at 60% water-holding capacity.

2.3. Determination of $^{14}CO_2$ losses

The flasks were removed at intervals of 4, 8, 12, 16 and 20 days and the $^{14}CO_2$ trapped in NaOH was analysed for ^{14}C by liquid scintillation counting (LSC).

[1] 1 Ci = 3.7 × 10^{10} Bq.

2.4. Determination of extractable residues

At different incubation intervals, air-dried samples (50 g) were extracted for 24 h with 150 mL of methanol at a rate of 5–6 cycles per hour in a Soxhlet extraction apparatus. The soil surface was covered with glass wool to help prevent the loss of soil particles. The extract was concentrated to 20 mL using a rotary evaporator (Rotavapor RE-120, Büchi, Switzerland). Aliquots of each extract were analysed for ^{14}C.

2.5. Determination of total and bound residues

Soil samples before extraction (extractable and bound residues) and after extraction (bound residues) were air-dried and 0.5 g subsamples were combusted to determine ^{14}C.

2.6. Organic matter fractionation

Twenty-gram portions of the soil incubated for 4, 8, 12, 16 and 20 days were shaken with 100 mL of NaOH + $Na_4P_2O_7$ (0.1N and 0.1M, respectively) solution for one hour, the mixture was centrifuged, and the supernatant (alkali extract) collected. A portion of the alkali extract was acidified to pH 2 with H_2SO_4; this was followed by centrifugation. The residue containing humic acid (HA) was then dissolved in 0.1N NaOH; the supernatant contained the fulvic acid (FA) fraction. The FA and HA solutions were analysed for ^{14}C as described below. The radioactivity in the humin fraction was obtained by subtracting the amounts of radioactivity in HA + FA from the total radioactivity bound in soil.

2.7. Estimation of microbial biomass

Microbial biomass was estimated by the fumigation technique of Jenkinson [24] and Jenkinson and Powlson [25].

2.8. Determination of radioactivity

Aliquots of extracts and dried soil samples were combusted in a Packard sample oxidizer (Model 306) to $^{14}CO_2$. This was then absorbed in and mixed with appropriate volumes of Carbosorb 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 $^{14}CO_2$ was mixed with 1 mL distilled water and 16 mL Quickszint-212 (Koch Light Laboratories Ltd, Federal Republic of Germany) in a scintillation vial and subjected to scintillation counting. The external standardization technique was used to correct for quenching.

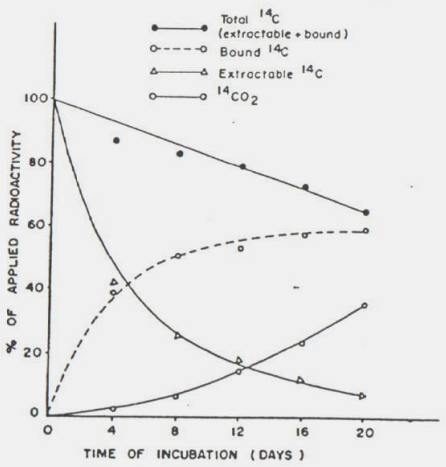

FIG. 1. Distribution of ^{14}C in $^{14}CO_2$ and in extractable and bound residues of ^{14}C-carbofuran in soil.

3. RESULTS AND DISCUSSION

The distribution of ^{14}C in $^{14}CO_2$ and in extractable and bound residues from ^{14}C-carbofuran in soil is shown in Fig. 1. The data presented indicate that after 20 days, 35.6% of the applied radioactivity was mineralized to $^{14}CO_2$. Further, the data show that there was a sharp decrease in the amount of extractable residues of ^{14}C-carbofuran over an incubation period of 20 days. After four days, the extractable ^{14}C-residues amounted to 41% of the applied radiocarbon. This decrease in extractable residues went in parallel with an increase in the formation of soil-bound ^{14}C-residues. At the end of the experiment, the extractable residues were 7%, the bound ^{14}C-residues were 59% and $^{14}CO_2$ evolution was 35.6% of the applied ^{14}C. The extensive binding of carbofuran to soil seems to be similar in behaviour to that of other methylcarbamate insecticides. For carbaryl (1 naphthyl-N-methylcarbamate), applied at 2 ppm to five soils, bound residues ranging from 17% to 57% were found, depending on soil type [18]. The amount of binding is thus a function of soil type, pesticide concentration, time and other parameters.

Table I shows the distribution of ^{14}C in organic matter fractions. After four days of incubation, 31.79%, 14.40% and 53.81% of the bound ^{14}C was

TABLE I. DISTRIBUTION OF ^{14}C FROM ^{14}C-LABELLED CARBOFURAN IN SOIL ORGANIC MATTER FRACTIONS[a]

Incubation period (days)	Percentage of applied radioactivity		
	Humic acid[b]	Fulvic acid[b]	Humin[c]
4	12.54 (31.79)	5.68 (14.40)	58.64 (53.81)
8	17.60 (31.26)	13.92 (24.73)	39.57 (44.01)
12	20.23 (35.44)	13.92 (24.40)	36.95 (40.16)
16	21.21 (35.97)	16.88 (28.62)	30.86 (35.41)
20	22.39 (38.23)	26.04 (44.45)	17.56 (17.23)

[a] Figures in parentheses indicate the percentage of bound ^{14}C in different fractions.

[b] Estimated bound residues.

[c] Total bound residues, alkali extractable.

present in humic acid, fulvic acid and humin, respectively. With prolonged incubation, the amount of ^{14}C increased in humic acid and fulvic acid, but it decreased in humin. For fulvic acid the increase was stronger than for humic acid. After 20 days of incubation, the percentage of bound ^{14}C in humic acid, fulvic acid and humin was 38.23%, 44.45% and 17.23%, respectively. Of the applied ^{14}C, 22.39%, 26.04% and 17.56% were incorporated into humic acid, fulvic acid and humin, respectively.

From the results presented in Table I, it can be seen that most of the bound ^{14}C-residues of carbofuran (53.81%) are in the humin fraction after 4 days of incubation; they are not extractable with alkali. However, it seems that later these bound residues are released from humin and are incorporated into humic acid and fulvic acid fractions. The relatively sharper increase of ^{14}C in fulvic acid between 16 and 20 days indicates that the residues thus released are concentrated in this fraction which is comparatively more labile. The recovery of high amounts of ^{14}C in fulvic acid may have an important bearing on its bioavailability, since fulvic acid is considered to be the dominant soluble organic matter fraction present in soil under field conditions [26].

Table II shows the amount of ^{14}C in microbial biomass. The unfumigated soil produced 230 525 dis/min per 50 g soil during 10 days of incubation.[2]

[2] 60 dis/min = 1 Bq.

TABLE II. AMOUNT OF ^{14}C IN MICROBIAL BIOMASS OF SOIL INCUBATED FOR 20 DAYS WITH ^{14}C-CARBOFURAN

Radioactivity (dis/min per 50 g soil)				Biomass ^{14}C (%)	
Evolved as $^{14}CO_2$[a]					
Unfumigated soil (A) (0–10 days)	Fumigated soil (B) (0–10 days)	Flush of decomposition[a] (B – A)	^{14}C in microbial biomass[b] (F × 2)	% of applied ^{14}C	% of residual ^{14}C
230 525 (10.38%)	396 891 (17.87%)	166 366 (7.49%)	332 731	14.99	27.18

[a] Figures in parentheses indicate the percentage of applied ^{14}C.

[b] The value of k was taken as 0.5.

Since the soil had been previously incubated for 20 days, the total incubation period for this soil is 30 days. The loss of ^{14}C from this soil amounted to 10.38% of the ^{14}C initially added. The loss of ^{14}C from fumigated soil was 72% higher than that from unfumigated soil. Calculations for biomass showed that 14.99% of the added ^{14}C was in this fraction. Of the residual ^{14}C, 27.18% was in microbial biomass. Thus the microbial population mineralized about 35.6% of the applied ^{14}C-carbofuran to CO_2 and incorporated 15% of it into the cellular mass. The rest of the ^{14}C was either in the form of carbofuran and its metabolites or in other components of soil organic matter.

The results presented in this study show that carbofuran is rapidly metabolized and, after 20 days of incubation, at least 36% of the applied chemical is passed through a microbial metabolic system.

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