PANEL PROCEEDINGS SERIES

QUANTIFICATION, NATURE AND BIOAVAILABILITY OF BOUND ^{14}C-PESTICIDE RESIDUES IN SOIL, PLANTS AND FOOD

PROCEEDINGS OF THE FINAL 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
AND HELD IN GAINESVILLE, FLORIDA,
FROM 25 TO 29 MARCH 1985

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1986

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

F. AZAM, A. HUSSAIN, K.A. MALIK
Nuclear Institute for Agriculture and Biology,
Faisalabad, Pakistan

Abstract

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

The availability of soil-bound ^{14}C-residues of malathion to maize plants and microbes was investigated under laboratory conditions. Maize plants were grown in clay loam soil treated with ^{14}C-malathion and it was found that 2.5% of the total bound ^{14}C and 6.6% of the freshly added ^{14}C was removed by the maize plants. In both cases the shoots contained more radioactivity than the roots and the uptake rate from freshly treated soil was three times higher than from soil containing bound residues. The analysis of the residual soil after harvesting the plants showed that 6.17% and 12.02% of the residual ^{14}C from bound and freshly applied malathion, respectively, could be extracted. This indicates that a part of the bound ^{14}C-residues was released during plant growth. The results further indicate that microbes can incorporate bound residues into their cellular mass more effectively than the plants and that microbial biomass can be used as an index for studying the bioavailability of agrochemicals applied to soil. In the soil containing bound ^{14}C-residues, no malathion was detected by high-temperature distillation and gas chromatography. When the methanol-extractable ^{14}C-residues were analysed with a flame photometric detector and gas chromatography, an unidentified compound was found which did not correspond to either malathion or malaoxon.

1. INTRODUCTION

Malathion (S-1,2-di(ethoxycarbonyl) ethyl 0-0-dimethyl phosphorodithioate) is an important selective insecticide used in the control of various pest insects; it is a widely used organophosphorus pesticide with low mammalian toxicity. With a view to environmental safety, the insecticide has been extensively studied regarding its metabolism in mammals, insects, fish, microbes, plants, foodstuff and soil [1–8]. However, no published information is available on the environmental fate of soil-bound residues of malathion. In a previous publication, we presented the results of a study on the formation of bound residues of malathion in clay loam soil under laboratory conditions [9]. It was shown that ^{14}C-malathion rapidly decomposed to $^{14}CO_2$, with a loss of 56% after 12 days of incubation. Bound and extractable residues amounted to 38% and 6%, respectively, of the applied dose.

In the present study, the uptake of soil-bound ^{14}C-malathion residues by maize plants and microbes is described, as well as the characterization and

identification of soil-bound ^{14}C-residues and of extractable residues of ^{14}C-malathion.

2. MATERIALS AND METHODS

2.1. Chemicals

Malathion (S-1,2-di(ethoxycarbonyl) ethyl 0-0-dimethyl phosphorodithioate) labelled at the 2,3 position of diethyl maleate (spec. act. 45.5 μCi/mg; 15 mCi/mmol)[1] was purchased from Amersham International, UK. Radiopurity was 98% as checked by thin-layer chromatography (TLC). Analytical grade malathion and malaoxon were obtained from American Cyanamid Co., Princeton, NJ. All other chemicals were of analytical grade and all solvents were reagent grade, freshly distilled before use.

2.2. Soil

The soil used was clay loam (organic matter 1.8%, sand 54%, silt 28%, clay 18%, pH 7.9); it was collected from NIAB fields and was stored before use at room temperature under moist conditions.

2.3. Preparation of soil-bound ^{14}C-malathion residues

Bound residues were produced by treating the triplicate moist soil samples (1250 g) with labelled (18.75 μCi) and unlabelled malathion in plastic pots to give an insecticide concentration of 6 mg/kg. The solvent was evaporated and the soil was thoroughly mixed. The soil was incubated for 30 days in the dark at 30 ± 2°C and then exhaustively extracted with methanol. Residual methanol from the extracted soil was allowed to evaporate by air-drying the sample. The bound ^{14}C-residues were determined by combustion of the air-dried soil to $^{14}CO_2$. The extracted soil was used for experiments on the bioavailability of bound ^{14}C-malathion residues to maize plants.

2.4. Plant uptake of soil-bound ^{14}C-malathion residues

The plant experiment was conducted in specially designed pots (PVC, 0.8 kg of soil per pot, Fig. 1), which permitted an air-tight separation of the root atmosphere. Two soil treatments, each with three replicates, were compared:

Treatment 1: Methanol-extracted soil containing bound ^{14}C-residues
Treatment 2: Soil with freshly added ^{14}C-malathion.

[1] 1 Ci = 3.7×10^{10} Bq

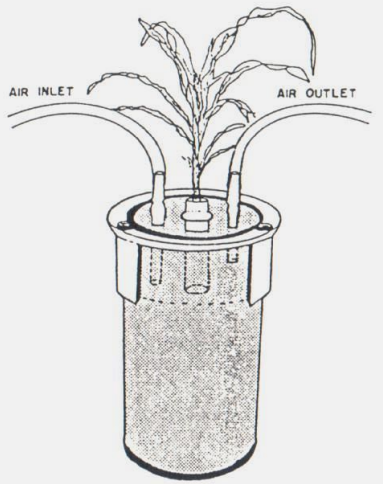

FIG. 1. Experimental pot used for studying the uptake of ^{14}C-labelled organic chemicals from soil.

Maize (*Zea mays* L., var. Neelum) was chosen as the experimental plant. Germinated seeds were planted into the pre-treated soil. The pots were irrigated daily or as necessary to keep the soil moisture at about field capacity. The soil was aerated and the CO_2, including $^{14}CO_2$ evolved from the root and soil respiration, was absorbed in 10% NaOH solution.

The plants were grown in the greenhouse with day and night temperature fluctuations of 34°C and 27°C, respectively. After 21 days the plants were harvested by cutting them 1 cm above the soil surface. Roots were removed from the soil and washed with distilled water. Portions of the dried plant tissue and residual soil were analysed for ^{14}C.

Portions of the soil remaining from treatments 1 and 2 were exhaustively extracted with methanol to determine extractable and bound radioactivity. The residual soil was also extracted with sodium hydroxide and sodium pyrophosphate (0.1N and 0.1M, respectively) to determine the distribution of ^{14}C in organic matter fractions [9].

2.5. Determination of extractable and bound residues

Air-dried samples of the soil were extracted for 24 h in a Soxhlet extraction apparatus as described in an earlier study [9]. The extract was concentrated and aliquots of each extract were analysed for their radioactivity. Extracted soil was air-dried to remove residual solvent, and 0.5 g subsamples were subjected to combustion to determine residual bound radioactivity.

2.6. Estimation of microbial biomass

Microbial biomass was estimated by the fumigation technique of Jenkinson and Powlson [10]. Fifty-gram portions of the soil (after removing the plants) were fumigated in duplicate and incubated at 30°C for 10 days in 250 mL Erlenmeyer flasks provided with glass cups containing 10% NaOH solution for absorbing $^{14}CO_2$. Untreated soil was also incubated. An aliquot of NaOH containing $^{14}CO_2$ was mixed with Quickszint-212 (Koch Light Laboratories Ltd. Federal Republic of Germany) in a scintillation vial and subjected to liquid scintillation chromatography. Biomass ^{14}C was calculated by using the formula B = F/K, where B is the biomass ^{14}C, F is the flush of decomposition ($^{14}CO_2$ evolved from fumigated soil minus that evolved from untreated soil during ten days of incubation) and K is the fraction of biomass mineralized to CO_2. The value of K was taken as 0.5 [11].

2.7. Determination of radioactivity

Aliquots of solutions and soil samples were combusted in a Packard oxidizer (Model 306) to CO_2. The $^{14}CO_2$ of the samples was determined in a Packard Tri-Carb liquid scintillation spectrometer (Model 3320). The external standardization technique was used for quench correction.

2.8. Analysis of bound ^{14}C-residues

An air-dried soil sample (80 mg) containing bound residues was subjected to the high-temperature distillation (HTD) technique under helium atmosphere to release the bound residues. The solvents used in the traps were methanol (trap I), acetone/hexane (trap II) and oxisorb (trap III, $^{14}CO_2$ trap). The trapping solutions were purified and then analysed by gas chromatography (GC).

2.9. Gas chromatography

2.9.1. Bound residues

For bound residue analysis the gas chromatograph used was a Varian Model 6000, fitted with a thermionic detector and interfaced with a Varian 404 data system. The column was a 1.8 m × 2 mm i.d. glass tube packed with 4% SE-30 6% QF-1. Helium was the carrier gas (40 mL/min) and the column, detector and injector temperatures were 190°C, 300°C and 220°C, respectively.

2.9.2. Extractable residues

A Perkin-Elmer (Model 3920) gas chromatograph, equipped with a flame photometric detector (FPD) that simultaneously monitors gas chromatographic effluents containing phosphorus (526 nm filter) on one channel and sulphur (394 nm filter) on the other, was used in the analyses for extractable residues.

A borosilicate glass column, 2 m × 2 mm i.d., filled with 5% OV-17 (wt/wt) on 80–100 mesh Chromosorb W, acid washed, was used. The carrier gas was nitrogen, adjusted to a flow rate of 30 mL/min. The column was heated isothermally at 150°C for 2 min after injection of a sample; then the temperature was raised at a rate of 16°C/min and held at 250°C for 8 min. The injector and detector temperatures were 200°C and 250°C, respectively. Hydrogen and air flowing through the detector were adjusted to 70 mL/min and 110 mL/min, respectively. The detector signals were recorded with a Hitachi Model 056 dual channel recorder at 1 m full-scale deflection.

The gas chromatographic analysis of the reference malathion and malaoxon (0.1% solution) was performed by injecting 0.5 μL into the column. The methanol extract was concentrated under a stream of nitrogen to 1 mL, and 8 μL were injected into the GC column for analysis.

Identification was achieved by comparing the GC retention times with those of authentic standards.

3. RESULTS AND DISCUSSION

3.1. Uptake of soil-bound ^{14}C-malathion residues

3.1.1. Maize

After a soil incubation of 30 days, 29.74% of the total applied ^{14}C was found to remain in the soil, the rest had been mineralized to $^{14}CO_2$. By exhaustive methanol extraction, 10% of the residual ^{14}C was extractable and 90% was bound.

Table I summarizes the results obtained for the uptake of bound and freshly applied ^{14}C-malathion residues from soil by maize plants. It was found that 2.45% of the total soil-bound ^{14}C and 6.59% of the freshly added ^{14}C was removed by maize plants. In both cases the shoots contained more radioactivity than the roots. The uptake from freshly treated soil was three times more than the uptake from soil containing only bound residues. The residual

TABLE I. UPTAKE OF BOUND AND FRESH ^{14}C-RESIDUES FROM SOIL BY MAIZE PLANTS[a]

Treatment	Roots	Shoots	Residual in soil	Roots + shoots + soil	Unaccounted ^{14}C[b]
1	0.19	2.26	89.27	91.72	8.28
2	1.97	4.62	31.02	37.60	62.40

[a] Percentage of the bound (treatment 1) or freshly added (treatment 2) ^{14}C.

[b] Unaccounted ^{14}C, probably lost as $^{14}CO_2$.

soil radioactivity after plant harvest amounted to 89.27% and 31.02% in treatments 1 and 2, respectively. During the 21 days of the plant experiment, the loss of ^{14}C was 8.28% from the soil containing bound residues and 62.4% from the soil treated with ^{14}C-malathion immediately before the plant test.

After the plant test, the soil was extracted with methanol to determine the relative distribution of residual activity in extractable and bound fractions. The results indicate that 6.17% and 12.02% of the residual ^{14}C from bound (treatment 1) and freshly applied (treatment 2) malathion, respectively, were extractable. This indicates that a part of the bound ^{14}C-residues was converted to an extractable form during plant growth.

Portions of the remaining soil were also analysed for the distribution of ^{14}C in organic matter fractions. The humic acid fraction contained 14.89% and 6.66%, the fulvic acid fraction contained 2.17% and 5.50%, and the humin fraction contained 82.94% and 87.84% of residual ^{14}C in treatment 1 and treatment 2, respectively.

The results reported here are in accordance with earlier studies [12–21] and support the view that the soil-bound residues can become available to plants, although the uptake was below 2.5% of the total soil-bound ^{14}C. The availability to plants of ^{14}C from soil containing bound residues is considerably lower than that from soil containing freshly added ^{14}C-malathion.

3.1.2. Microbial biomass

Freshly applied and soil-bound ^{14}C-malathion residues were incorporated into microbial biomass. Soil analysis after harvesting the plants showed that 4.40% and 4.30% of the ^{14}C present initially as bound residues and in freshly applied ^{14}C-malathion, respectively, was transformed into microbial biomass. On the basis of residual ^{14}C in soil after harvesting the plants, it was found that

4.93% and 13.06% of the ^{14}C in treatment 1 and treatment 2, respectively, was in biomass. A comparison of these results with those presented in Table I shows that microbes can incorporate bound residues into their cell mass more effectively than plants. In the case of freshly applied malathion, more ^{14}C was taken up by the plants than by microbial biomass, due to the fact that newly synthesized ^{14}C-labelled microbial biomass undergoes rapid turnover and resynthesis. The results suggest that microbial biomass can be used as an index for studying the bioavailability of agrochemicals. The benefit of using microbial biomass as an index is that its estimation is easier than the evaluation of bioavailability by plant growth experiments which are time consuming and for which specific precautions are needed.

3.2. Characterization of soil-bound ^{14}C-malathion residues by the HTD–GC technique

High-temperature distillation (HTD) was used for determining and chemically identifying the soil-bound residues of ^{14}C-malathion [22]. The amount of radioactivity in the combined material from traps I and II was 22.8% of the total radioactivity released by HTD. The radiocarbon which thermally decomposed to $^{14}CO_2$ was 33% (trap III); and 43% ^{14}C remained in the quartz tube and silicate residues. The total activity recovered was 13 050 dis/min per gram soil.[2] The distillates, subjected to various cleanup and extraction procedures, were analysed to determine the identity of the bound residues. It was not possible to detect malathion in any of the traps by GC analysis.

3.3. Characterization of extractable residues of ^{14}C-malathion by the FPD–GC technique

Gas chromatograms of the standard solutions of malathion and its oxygen analog (malaoxon) using the dual-channel FPD are shown in Fig. 2. The use of OV-17 as the stationary phase gave complete separation of the two components. The retention times for malathion and malaoxon were 11.2 min and 6.2 min, respectively.

Gas chromatographic examination of the methanol-soluble residues indicated the presence of an unidentified compound (Fig. 2). The unknown compound had a retention time of 10.6 min, which did not correspond to that of malathion or malaoxon. No attempts were made to identify other derivatives of malathion because of the unavailability of reference standards.

[2] 60 dis/min = 1 Bq

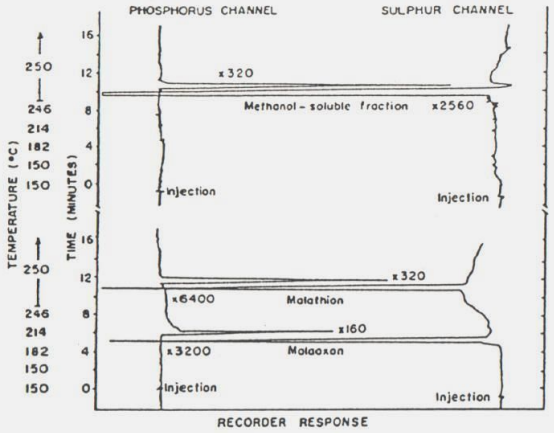

FIG. 2. Gas chromatograms of standard malathion, malaoxon and the methanol-soluble fraction.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. S.U. Khan of the Chemistry and Biology Research Institute, Agriculture Canada, Canada, for analysing the bound ^{14}C-residues of soil samples, and the American Cyanamid Co., Princeton, New Jersey, USA, for contributing analytical grade malathion and malaoxon.

REFERENCES

[1] KRUEGER, H.R., O'BRIEN, R.D., J. Econ. Entomol. **52** (1959) 1063.
[2] SHAFIQ, M.T., ENOS, H.F., J. Agric. Food Chem. **17** (1969) 1186.
[3] COOK, G.H., MOORE, J.C., J. Agric. Food Chem. **24** (1976) 631.
[4] LICHTENSTEIN, E.P., SCHULZ, K.R., J. Econ. Entomol. **57** (1964) 618.
[5] MATSUMURA, F., BOUSCH, G.M., Science **153** (1966) 1278.
[6] GARDNER, A.M., DAMICO, J.N., HANSEN, E.A., LUSTIG, E., STORHERR, R.W., J. Agric. Food Chem. **17** (1969) 1181.
[7] ANDEREGG, B.N., MADISEN, L.J., J. Agric. Food Chem. **31** (1983) 700.
[8] KONRAD, J.G., CHESTER, G., ARMSTRONG, D.E., Soil Sci. Soc. Am., Proc. 33 (1969) 259.

[9] HUSSAIN, A., AZAM, F., MALIK, K.A., "Studies on bound residues of ^{14}C-malathion in soil", Radiotracer Studies of Bound Pesticide Residues in Soil, Plants and Food (Rep. Res. Co-ordination Meeting Neuherberg, 1983), IAEA-TECDOC-306, IAEA, Vienna (1984) 17.
[10] JENKINSON, D.S., POWLSON, D.S., Soil Biol. Biochem. **8** (1976) 209.
[11] JENKINSON, D.S., Soil Biol. Biochem. **8** (1976) 203.
[12] SCHEUNERT, I., MARRA, C., VISVANATHAN, R., KLEIN, W., FORTE, F., Chemosphere **12** (1983) 483.
[13] FUHREMANN, T.W., LICHTENSTEIN, E.P., J. Agric. Food Chem. **26** (1978) 605.
[14] HELLING, C.S., KRIVONAK, A.E., J. Agric. Food Chem. **26** (1978) 1164.
[15] FÜHR, F., MITTELSTAEDT, W., J. Agric. Food Chem. **28** (1980) 122.
[16] KHAN, S.U., J. Agric. Food Chem. **28** (1980) 1096.
[17] ROBERTS, T.R., STANDEN, M.E., Pestic. Sci. **12** (1981) 285.
[18] WEISS, U.M., MOZA, P., SCHEUNERT, I., HAQUE, A., KORTE, F., J. Agric. Food Chem. **30** (1982) 1185.
[19] IMAI, Y., KUWATSUKA, S., J. Pestic. Sci. **9** (1984) 79.
[20] BULL, D.L., IVIE, G.W., MacCONNELL, J.G., GRUBER, V.F., KU, C.C., ARISON, B.H., STEVENSON, J.M., Van den HEUVEL, W.J.A., J. Agric. Food Chem. **32** (1984) 94.
[21] ANDEREGG, B.N., LICHTENSTEIN, E.P., J. Agric. Food Chem. **32** (1984) 610.
[22] KHAN, S.U., HAMILTON, H.A., J. Agric. Food Chem. **28** (1980) 126.