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Persistence of fungitoxicity of two triorganotin(IV) compounds in soil

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The persistence of triphenyltin chloride-triphenylphosphine oxide ($Ph_3SnCl \cdot Ph_3PO$) and diphenylbutyltin bromide ($Ph_2BuSnBr$) in unsterilized sandy loam soil maintained in the dark at 60% of its water-holding capacity and at 27 ± 2°C was studied over a period of 29 days. The percentage recovery of the compounds upon extraction with acetone immediately after application to soil was 6% for $Ph_3SnCl \cdot Ph_3PO$ and 9.8% for $Ph_2BuSnBr$. The half-lives of the compounds were 15 days for $Ph_3SnCl \cdot Ph_3PO$ and 14.2 days for $Ph_2BuSnBr$. After 29 days following application of the compounds in soil $< 8.3\ \mu g\ g^{-1}$ of $Ph_3SnCl \cdot Ph_3PO$ and $< 5.1\ \mu g\ g^{-1}$ of $Ph_2BuSnBr$ remained in soil compared with the starting concentration of 50 μg of each compound per gram soil. The two triorganotin compounds were evidently easily degraded and the compounds may be applied every 2–3 weeks in the field at a rate of at least 0.005–0.01 kg ha^{-1}.

Keywords: Triorganotin compounds, triphenyltin chloride·triphenylphosphine oxide, diphenylbutyltin bromide, persistence, fungitoxicity

INTRODUCTION

Biological activity or toxicity of a compound depends on its chemical structure and the organisms which are exposed to it.[1] Biological activity, however, does not necessarily imply that the chemical is also readily available to the target organism. The method of application and the type of formulation can influence the availability of an agrochemical to the target organism.[1] The biological availability of a compound in soil also depends on the soil properties and climatic conditions. All these factors influencing the biological activity and availability of pesticides in soil have been well reviewed.[2–4]

An important factor influencing the activity of pesticides in soil is the adsorption of pesticides by soil particles.[5] Harris[6,7] showed that biological activity in terms of LD_{50} values of the insecticides diazinon, dieldrin and heptachlor is a function of the organic carbon content in soil. A similar relationship was observed for the herbicides methabenzthiazuron and monolinuron.[8,9]

Persistence is a term which does not have a set meaning.[10] It can be understood in a chemical or biological sense as chemical or biological persistence. In this study, persistence refers to the amount of triorganotin(IV) compounds present in treated soil on successive intervals of time as measured by a modified bioassay method introduced by Richardson[11] and Munnecke.[12] The biological availability as measured by bioassay methods correlates satisfactorily with the rate of disappearance measured by chemical analysis.[13–15]

Studies using labelled compounds have shown that triphenyltin acetate degrades through di- and monophenyltin compounds to non-toxic inorganic tin residues.[16] Triphenyltin acetate on exposed plant parts can be decomposed photochemically.[17–19] Triphenyltin acetate in soil interstices, however, is degraded by microorganisms.[20] Bis(tributyltin) oxide is also degraded by micro-organisms in soil.[20]

Cenci and Cremoni[21] reported that triphenyltin acetate and hydroxide could not be detected by thin layer chromatography after 12–240 h in different soils. Using ^{113}Sn-labelled preparations, Massaux[22] reported that, within seven days, approximately 99% of triphenyltin chloride is mineralized in the ground. Barnes et al.,[19] however, used ^{14}C-labelled

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triphenyltin acetate and reported a chemical half-life of 140 days for the compound in soil. A constant linear rate of $^{14}CO_2$ evolution (0.44% day^{-1}) occurred up to day 80, at which time approximately one-third of the phenyl carbon had been released. The rate of $^{14}CO_2$ production fell thereafter to less than half of the initial rate (0.21% day^{-1}). Barug and Vonk[20] showed that bis(tributyltin) oxide has a relatively longer chemical half-life of approximately 15 and 20 weeks in unsterilized silt loam and sandy loam, respectively.

Suess and Eben[23] studied the adsorption characteristics of ^{14}C-triphenyltin acetate in two kinds of soil used for cultivation of hops. The tests showed that of the 478 μg ^{14}C-triphenyltin acetate added to 10 g of soil, 31.5% could be removed from the light soil and 35.6% from the heavy soil from three extractions with chloroform. There was no major difference in the decomposition rate of ^{14}C-triphenyltin acetate between light and heavy soil, but ^{14}C-triphenyltin acetate adsorbed to the soil decomposed faster than material which had previously been admixed. Studies on the persistence of organotin compounds using bioassay techniques, however, have not been published. Only one published study on the detoxification of bis(tributyltin) oxide by species of *Phialophora* using *Bacillus subtilis* as the test organism has hitherto been carried out.[24]

The aim of the present study was to investigate the persistence of two triorganotin(IV) compounds, viz. triphenyltin chloride·triphenylphosphine oxide ($Ph_3SnCl \cdot Ph_3PO$) and diphenylbutyltin bromide ($Ph_2BuSnBr$) in soil. The two compounds were selected on the basis of their good *in vitro* and *in vivo* antifungal activities. This study would demonstrate if the compounds are detoxified in soil, the time taken for the detoxification and hence the frequency with which these compounds should be applied in the field.

MATERIALS AND METHODS

Test fungus

Trichoderma viride was selected as the test organism for this study because earlier antifungal screening[25] showed that the fungus is sensitive to triphenyltin chloride·triphenylphosphine oxide (ED_{50} = 6.99 μg cm^{-3}) and diphenylbutyltin bromide (ED_{50} = 0.66 μg cm^{-3}).

Trichoderma viride was inoculated on 2% malt extract agar (MA) slants in 15 cm × 1 cm test-tubes each containing 5 cm^3 of the medium. All test-tubes were incubated at 27 ± 2°C for six days. Sterile distilled water (10 cm^3) from MaCartney bottles was poured into the culture tubes. The spores were suspended in this water by gently scraping the surface of the culture with a sterile blunt spatula. The spore suspension was returned to the MaCartney bottle and the contents were vigorously shaken with a Vortex Whirlmixer. The spore suspension was serially diluted to give 2 × 10^4 spores cm^{-3}.[12]

Preparation of bioassay plates

About 15 cm^3 of 2% MA was poured into 9-cm sterile plastic Petri plates and allowed to set. A 0.1 cm^{-3} aliquot of *T. viride* spore suspension containing 2 × 10^4 spores cm^{-3} was spread on each plate, referred to subsequently as a bioassay plate. Using a sterile cork borer no. 5, 7-mm diameter discs were then cut out from the centre of each bioassay plate to create a well in the centre of each plate. Bioassay plates were prepared 30 min before they were used.

Preparation of standard curves for $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$

Stock solutions of $Ph_3SnCl \cdot Ph_3PO$ at concentrations of 1000 μg cm^{-3} and 100 μg cm^{-3} were prepared. Concentrations of 1, 5, 10, 25 and 50 μg cm^{-3} in analytical-grade acetone were prepared from the two stock solutions and 0.05 cm^3 of each concentration was dispensed into the 7-mm wells of the bioassay plates in triplicate. Before the acetone in the wells evaporated off completely, 0.1 cm^3 of sterile distilled water was dispensed into each well to give concentrations of 0.5, 2.5, 5.0, 12.5 and 25.0 μg cm^{-3} respectively. All plates were incubated at 27 ± 2°C for 48 h. The diameters of the zone of inhibition of growth were measured in directions at right angles to each other for each plate. The standard curve for the compound was constructed by plotting the mean diameter of inhibition of growth minus the well diameter against log concentration of the compound.[12]

The procedure described above was repeated using $Ph_2BuSnBr$.

Soil treatment

The soil samples used in the study were obtained from field plots planted with 18-month-old black pepper (*Piper nigrum*) cuttings. The soil was classified as a sandy loam comprising 97% sand (that fraction with particles > 0.02 mm diameter) and 3% silt (that

fraction with particles < 0.02 mm diameter). The water-holding capacity (WHC) of the soil as a whole was 30%, the total nitrogen content 0.14% and the total organic carbon content 21.7%. The bacterial count in the soil held at 60% WHC was 1.96×10^7 organisms per g soil.

Soil previously air-dried overnight and sieved through < 2 mm wire mesh sieve was weighed into two lots of 300 g (oven-dry basis) each. One lot was mixed with compound $Ph_3SnCl \cdot Ph_3PO$ and the other with $Ph_2BuSnBr$ at the rate of 50 μg of either compound for every gram of soil used. For each compound 15 mg was dissolved in 10 cm^3 of analytical-grade acetone to which enough sterile distilled water was added to give 60% WHC when added to the soil. The organotin suspension was shaken thoroughly and added to the soil held in enamel trays. The organotin-incorporated soil was mixed thoroughly with a spatula. For each compound, 100 g (oven-dry basis) of the treated soil was dispensed into each of three replicate 250 cm^3 Erlenmeyer flasks. The flasks were covered with parafilm, weighed and then incubated at 27 ± 2°C in the dark for 29 days. The moisture content of the soil was maintained at 60% WHC throughout the experiment by adding sterile distilled water to compensate for any loss in weight of the flasks. The soil was bioassayed for the extractable $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ in the soil immediately after treatment (Day 0) and for that remaining 1, 2, 3, 4, 7, 14, 21 and 29 days after treatment.

Extraction and bioassay of $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$

On each sampling day, 1 g (oven-dry basis) of treated soil was removed from each flask into a 100 cm^3 Erlenmeyer flask and 5 cm^3 of analytical-grade acetone was added to the soil. The flasks were covered with parafilm and shaken for 1 h at 125 rpm in an orbital shaker. The extractant was filtered through Whatman No. 1 filter paper into a 50 cm^3 beaker. The acetone was allowed to evaporate off completely. The residue was redissolved in 0.5 cm^3 of analytical-grade acetone to which 0.5 cm^3 of sterile distilled water was added and mixed thoroughly. (0.1 cm^3) aliquots of this treated soil extract were dispensed into the wells cut out in the bioassay plates in duplicate. All plates were incubated at 27 ± 2°C for 48 h. The diameters of the zones of inhibition of growth were measured in directions at right angles to each other. The concentrations of the organotin fungicides in the soil were read off from the standard calibration curves constructed for each compound. These ranged from 3.0 ± 0.2 $\mu g\ g^{-1}$ (Day 0) to 0.5 ± 0.1 $\mu g\ g^{-1}$ (Day 29) for $Ph_3SnCl \cdot Ph_3PO$ and from 4.9 ± 0.4 $\mu g\ g^{-1}$ (Day 0) to 0.5 ± 0.4 $\mu g\ g^{-1}$ (Day 29) for $Ph_2BuSnBr$. Inasmuch as the recovery of the organotin from the soil by a single extraction with acetone was low as judged by the above data for Day 0, the actual concentration of the two compounds present in soil on each sampling day was corrected for by multiplying the extractable concentration as reflected from the bioassay result by the recovery factor of 50/3.0 and 50/4.9, respectively.

RESULTS

Calibration curves for $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$

The calibration curves for $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$, respectively, are given in Figs 1 and 2.

Bioassay for persistence of $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$

The bioassay method used here could detect as little as 0.5 $\mu g\ cm^{-3}$ of both the triorganotin(IV) compounds and it was thus possible to construct the decay curves for the compounds. Based on the assumption of constancy in the ratio of extractable to non-extractable organotin in each case, the concentrations of $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ in the soil, corrected for poor recovery as previously indicated, were plotted against the corresponding sampling days to yield the exponential decay curves depicted in Figs 3 and 4.

The half-lives of the compounds were obtained from the decay curves. $Ph_3SnCl \cdot Ph_3PO$ had a half-life of 15 days whereas $Ph_2BuSnBr$ had a half-life of 14.2 days. Degradation of $Ph_3SnCl \cdot Ph_3PO$ was rapid in the first 7 days and more gradual in the next 22 days. After 29 days of treatment, < 8.3 $\mu g\ g^{-1}$ and < 5.1 $\mu g\ g^{-1}$ of $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$, respectively, persisted in the soil.

DISCUSSION

The low extractability of the triorganotin(IV) compounds in loamy sand with 18% moisture content and 21.7% total organic carbon was probably due to

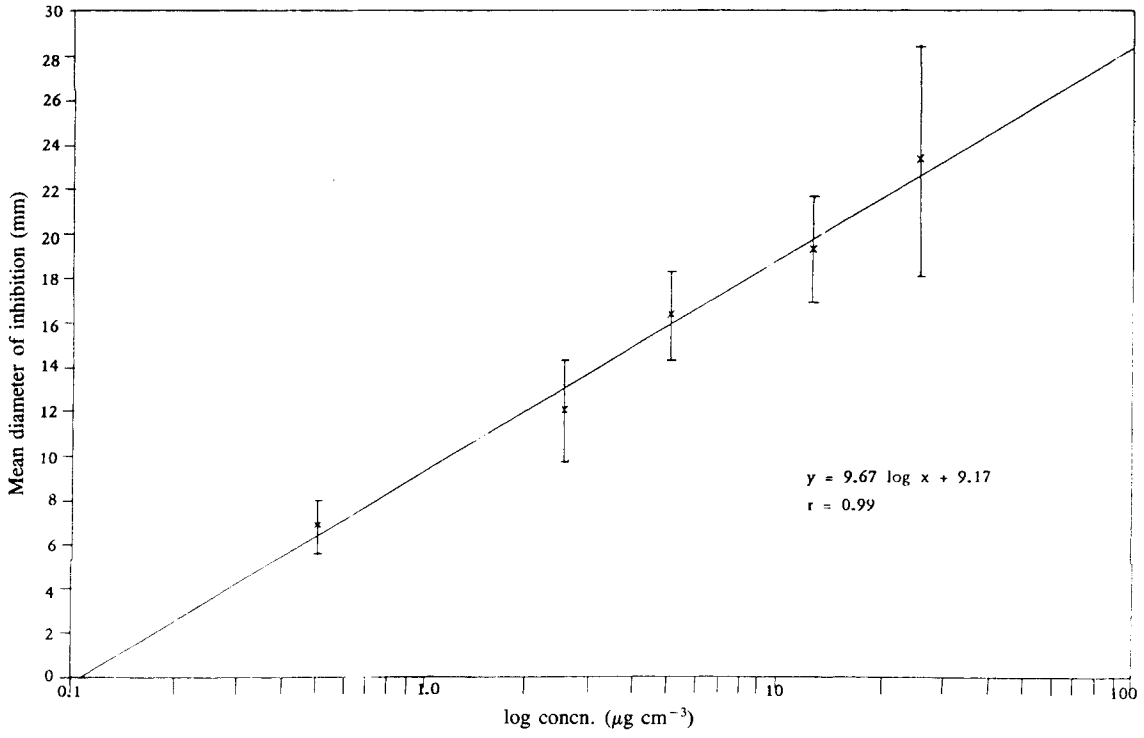

Figure 1 Calibration curve for $Ph_2SnCl \cdot Ph_3PO$ (vertical bars show standard deviation).

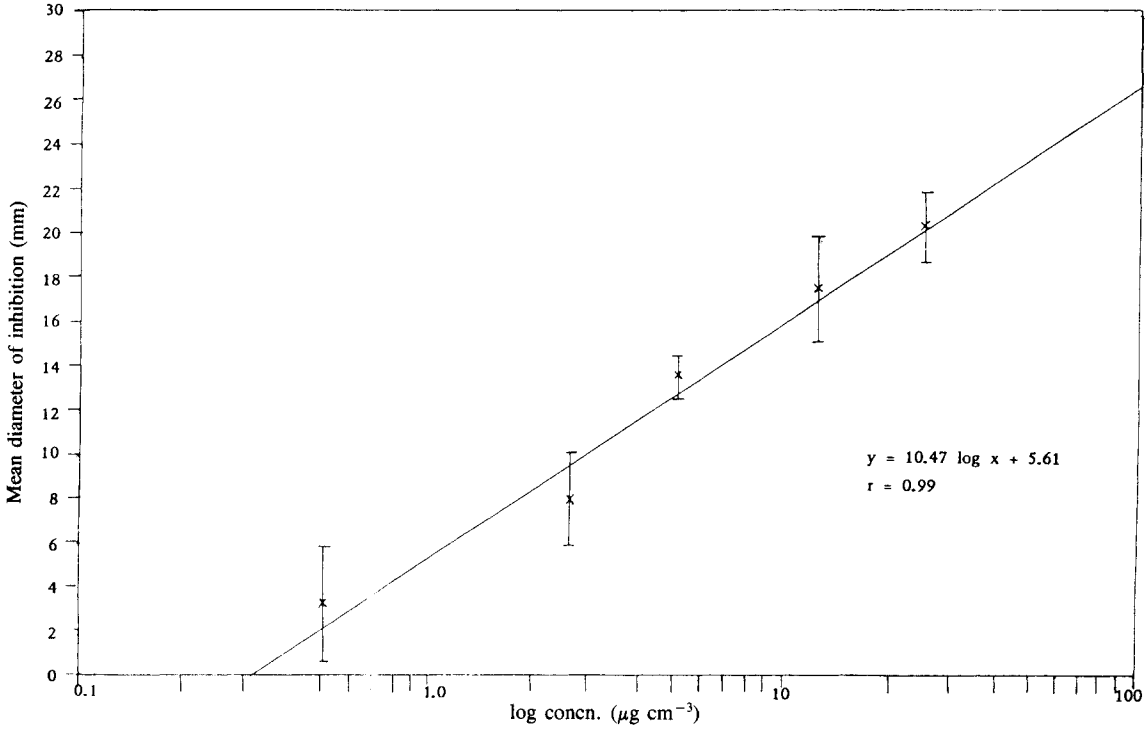

Figure 2 Calibration curve for $Ph_2BuSnBr$ (vertical bars show standard deivation).

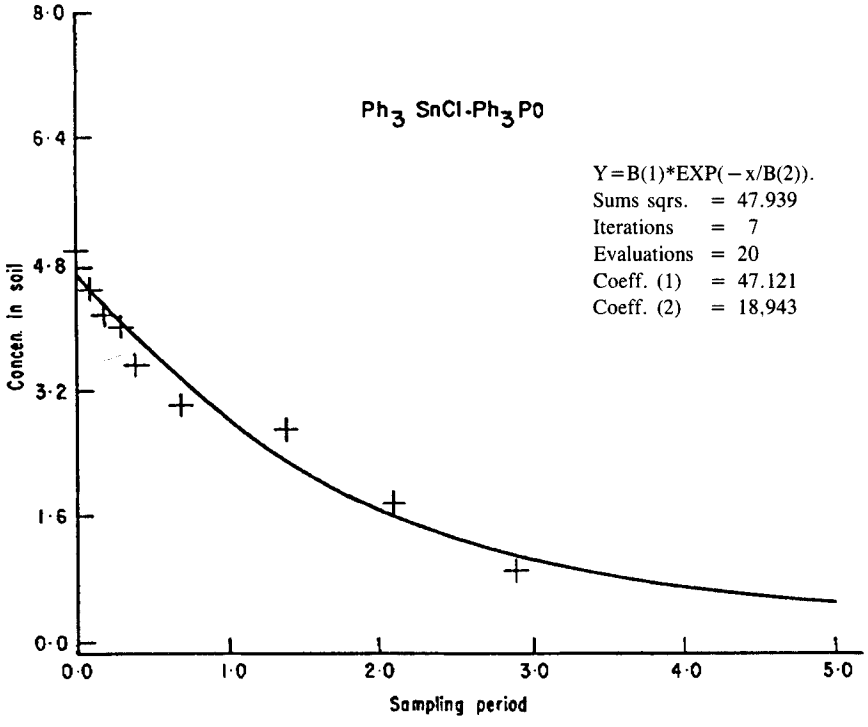

Figure 3 Degradation of $Ph_3SnCl \cdot Ph_3PO$ in soil.

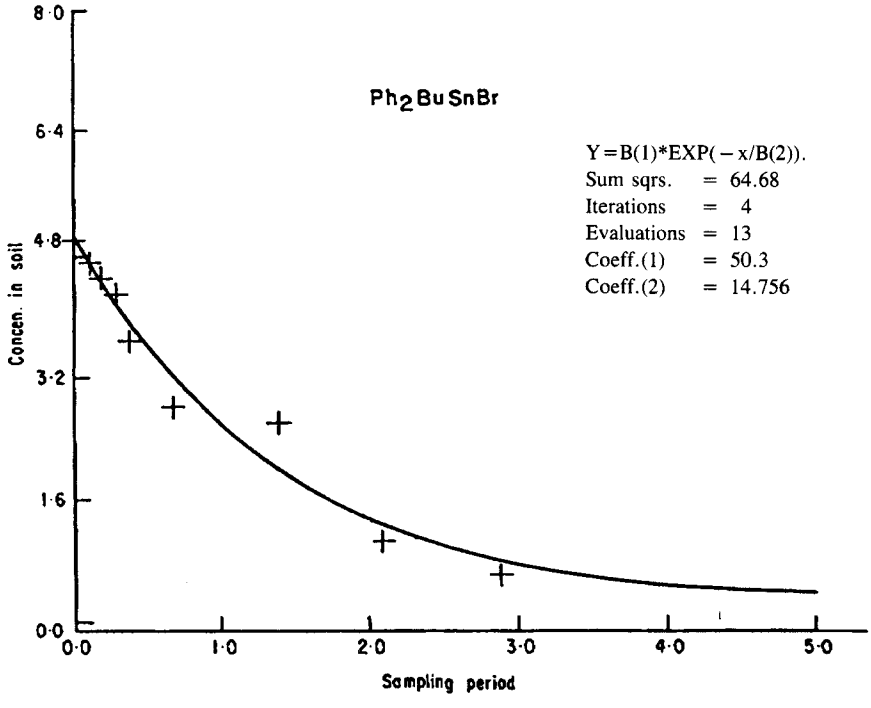

Figure 4 Degradation of $Ph_2BuSnBr$ in soil.

the high adsorption of the compounds onto soil particles. Barnes *et al.*[19] had similarly reported that following leaching only 20% of the original ^{14}C-triphenyltin acetate could be extracted from agricultural loam of the Stretham series VI. From non-perfused soil, however, 45% could be extracted in a comparable period. Hence, Barnes *et al.*[19] suggested that the adsorption of triphenyltin acetate onto soil particles is influenced by the moisture status of the soil. The low extractability of $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ in the present study could also be attributed to the fact that only a single extraction was attempted for each case with acetone.

The degradation curves obtained for the two compounds are similar to that obtained for triphenyltin acetate by Barnes *et al.*[19] and for bis(tributyltin) oxide by Barug and Vonk.[20] The breakdown of $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ was not preceded by a distinct lag period. This result suggests, most probably, a very rapid adaptation of the soil micro-organisms to the two compounds.

The bioassay method used in this experiment could detect up to 0.5 $\mu g\ cm^{-3}$ only of the triorganotin(IV) compounds. Hence, chemical assay is required to detect $< 0.5\ \mu g\ cm^{-3}$ of the compounds if the time taken for complete breakdown of the compounds is to be determined more precisely.

The half-lives of the two compounds (15.0 and 14.2 days, respectively) indicate that they are easily degraded, probably, by soil microorganisms. It can be further inferred that $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ can be applied every 2–3 weeks in the field at a rate of at least 0.005–0.01 kg ha^{-1}.

A simple bioassay method for the evaluation of the rate of inactivation of fungicides, mainly thiocarbohydrazone derivatives and copper 8-quinolinolate, was devised by Jackson and Kempton,[26] who showed that during the course of incubation of each 1% (w/w) soil/fungicide mixture, the quantity of active fungicide per unit weight of soil was gradually reduced. For the thiocarbohydrazone derivatives, the results were explicable in terms of biological detoxification rather than chemical degradation.

Unlike the method used by Jackson and Kempton,[26] the bioassay technique used in the present study allowed for quantification of the inhibition produced by the fungicide still persistent in the soil. The advantages of using a very sensitive fungus such as *Trichoderma viride* are also evident. In the present study it is apparent that the loss of biological activity with time and therefore of the decreasing presence of $Ph_3SnCl \cdot Ph_3PO$ and $Ph_2BuSnBr$ in the respective acetone extracts is the result of chemical degradation of the compounds in the soil rather than the consequence of a variable pattern in the soil adsorption characteristics. The conclusion is based on the observation that about 29 days of incubation of the fungicide/soil mixture was required before almost complete loss of fungitoxicity occurred. The results obtained do not however permit any firm conclusions to be drawn on the mode of breakdown of the organotin compounds in the soil.

CONCLUSIONS

The bioassay technique used in the prsent study could detect as little as 0.5 $\mu g\ cm^{-3}$ of the two triorganotin(IV) compounds and is therefore considered to be reliable.

The half-lives of the two compounds are 15 days for triphenyltin chloride·triphenylphospine oxide and 14.2 days for diphenylbutyltin bromide.

Twenty-nine days after application of the compounds in soil, $< 8.3\ \mu g$ $Ph_3SnCl \cdot Ph_3PO$ and $< 5.1\ \mu g$ $Ph_2BuSnBr$ per g soil, respectively, remain in soil compared with the initial concentration of 50 μg of either compound per g soil.

About 80–90% of the compounds are degraded within 29 days after application in soil.

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REFERENCES

1. Guth, J A, Gerber, H R and Schlaepfer, T H In: *Proc. 1977 British Crop Protection Conf. — Pests and Diseases*, 1977, pp 961–971
2. Edwards, C A In: *Organic Chemicals in the Soil Environment*, vol 2, Goring C A I and Hamaker J W (eds.), 1972, pp 513–568
3. Upchurch, R P In: *Organic Chemicals in the Soil Environment*, vol 2, Goring C A I and Hamaker J W (eds), 1972, pp 443–512
4. Burns, R G In: *Soil Biochemistry*, Paul E A and McLaren A D (eds), 1975, pp 103–141
5. Adams, R S Jr *Residue Rev.*, 1973, 47: 1
6. Harris, C K J *Econ. Entomol.*, 1972, 65: 8
7. Harris, C K *Ann. Rev. Entomol.*, 1972, 17: 177

8. Ashton, F M and Sheets, T J *Weeds*, 1959, 7: 38
9. Pestemer, W *Weed Res.*, 1976, 16: 357
10. Frehse, H *Proc. British Crop Protection Cooperation Symp. — Persistence of Insecticides and Herbicides*, 1976, pp 1–39
11. Richardson, L T *Can. J. Bot.*, 1954, 32: 335
12. Munnecke, D E *Phytopathology*, 1958, 48: 61
13. Guth, J A, Geisobuehler, H and Ebner, L *Meded. Rijksfaculteit Landbouweten-Schapper*, 1969, 34: 1027
14. Ederbe, D O and Gerber, H R *Arch. Environ. Contam. Toxicol.*, 1976, 4: 101
15. Walker, A *Pestic. Sci.*, 1977, 8: 359
16. Evans, C J *Tin and Its Uses*, 1974, 100: 3
17. Graham-Bryce, I J and Briggs, G G *RIC Revies*, 1970, 3: 88
18. Freitag, K D and Bock, R *Pestic. Sci.*, 1974, 5: 731
19. Barnes, R D, Bull, A T and Poller, R C *Pestic. Sci.*, 1973, 4: 305
20. Barug, D and Vonk, J W *Pestic. Sci.*, 1980, 11: 77
21. Cenci, P and Cremoni, B *Industria Succarifera Italiana*, 1969, 62: 313
22. Massaux, F *Caf Cacao Thé*, 1971, 15: 221
23. Suess, A and Eben, C H *Pflanzenschutz.*, 1973, 80: 288
24. Sutter, H P and Carey, J K *Int. Biodetn. Bull.*, 1986, 22: 195
25. Wickneswari, R PhD Thesis, University of Malaya, Kuala Lumpur, Malaysia, 1988
26. Jackson, E and Kempton, A G *Int. Biodetn. Bull.*, 1975, 11: 109