nodules. The import of carbon in the phloem of plants inoculated by Hup^- was higher than the Hup^+ strains. In pea, Hup^+ nodules used less carbon assimilate per unit of C_2H_2 reduction compared to the Hup^- nodules[25]. Though in some crops, no positive correlation between Hup^+ character and symbiotic efficiency has been observed, a majority of the evidence supports the view that H_2 recycling capability benefits legume growth[26]. Among the precautions that are necessary to conduct comparative evaluation of Hup^+ and Hup^- strains one is the oxidation in Hup^+ strain that should be coupled to ATP synthesis. The bacteroids of Hup^+ strain Vp 1 used in the present investigation had nearly two-fold higher ATP level compared to the Hup^- mutant[15]. Thus the beneficial effects of H_2-recycling ability of rhizobia are due to reduced photosynthate requirement of nodules.

1. Dixon, R. O. D., *Arch. Microbiol.*, 1972, **85**, 193.
2. Phillips, D. A., *Annu. Rev. Plant Physiol.*, 1980, **31**, 29.
3. Schubert, K. R., Jennings, N. J. and Evans, H. J., *Plant Physiol.*, 1978, **61**, 398.
4. Pahwa, K. and Dogra, R. C., *Arch. Microbiol.*, 1981, **129**, 380.
5. Rainbird, R. M., Atkins, C. A., Pate, J. S. and Stanford, P., *Plant Physiol.*, 1983, **72**, 308.
6. Nelson, L. M. and Child, J. J., *Can. J. Microbiol.*, 1981, **27**, 1028.
7. Dadarwal, K. R., Grover, R. and Tauro, P., *Arch. Microbiol.*, 1982, **133**, 303.
8. Eisbrenner, G. and Evans, H. J., *Annu. Rev. Plant. Physiol.*, 1983, **34**, 105.
9. Drevon, J. J., Godfroy, A., Heckmann, M. D., Ollat, N. and Payre, H., in *Nitrogen Fixation: Hundred Years After*, Proceedings of the 7th International Congress on Nitrogen Fixation, (eds. Bothe, H., de Bruijn, F. J. and Newton, W. E.), Gustav Fischer, Stuttgart, 1988, p. 584.
10. Gordon, A. J., Ryle, G. J. A., Mitchell, D. F. and Powell, C. E., *J. Exp. Bot.*, 1982, **33**, 241.
11. Koch, K. E. and Schrader, L. E., *Plant Physiol.*, 1984, **75**, 1040.
12. Kouchi, H. and Akao, S., *J. Exp. Bot.*, 1986, **37**, 985.
13. Wasfi, M. and Prioul, J. L., *Physiol. Plant*, 1986, **66**, 481.
14. Garg, N., Garg, R. P. and Nainawatee, H. S., *Indian J. Exp. Biol.*, 1990, **28**, 427.
15. Garg, N. and Nainawatee, H. S., *Phytochemistry*, 1989, **28**, 2913.
16. Dahiya, J. S. and Khurana, A. L., *Plant Soil*, 1981, **83**, 299.
17. Sloger, C., *Plant Physiol.*, 1969, **44**, 1666.
18. Hooda, R. S., Sheoran, I. S. and Singh, R., *Ann. Appl. Biol.*, 1989, **114**, 367.
19. Bray, G. A., *Anal. Biochem.*, 1960, **1**, 279.
20. Anonymous, Official Methods of Analysis, Association of Official Agricultural Chemists, 11th edn, Washington, DC, 1970, p. 16.
21. Minchin, F. R. and Pate, J. S., *J. Exp. Bot.*, 1973, **24**, 295.
22. Layzell, D. B., Rainbird, R. M., Atkins, C. A. and Pate, J. S., *Plant Physiol.*, 1979, **64**, 888.
23. Drevon, J. J., Frazier, L., Russell, S. A. and Evans, H. J., *Plant Physiol.*, 1982, **70**, 1341.
24. Rainbird, R. M., Atkins, C. A. and Pate, J. S., *Current Perspectives in Nitrogen Fixation* (eds. Gibson, A. H. and Newton, W. E.), North Holland, Elsevier, 1981, p. 376.
25. Skrdleta, V., Lisa, L. and Nemoova, M., *Folia Microbiol.*, 1987, **32**, 234.
26. Evans, H. J., Harker, A. R., Papen, H., Russel, S. A., Hanus, F. J. and Zuber, M., *Annu. Rev. Microbiol.*, 1987, **41**, 335.

ACKNOWLEDGEMENT. One of the authors (NG) thanks the Council of Scientific and Industrial Research, New Delhi, for a fellowship.

Received 15 October 1990; revised accepted 17 June 1991

Bioavailability of soil-bound residues of DDT and HCH to earthworms

Anita Verma and M. K. K. Pillai

Department of Zoology, University of Delhi, Delhi 110 007, India

We report bioaccumulation of non-extractable soil-bound residues of DDT and HCH by earthworms. Earthworms also facilitate steady mobilization of soil-bound residues to readily bioavailable labile forms.

THE formation of soil-bound residues of certain pesticides has been well documented in recent years[1-3]. Bound residues are essentially those residues that remain in soil after exhaustive solvent extraction and are detected by combustion or strong hydrolytic and pyrolytic techniques[3]. Whether the soil-bound residues of pesticides remain inert in the soil or become bioavailable is yet to be ascertained fully, though there are reports that they can be released by micro-organisms[4,5], may be taken up by plants[6,7] and animals[6,8,9]. Dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) constitute more than 70% of the total pesticides currently used in India. Recent studies have shown for the first time that they form bound residues in tropical soil[10] but their possible environmental hazard is not known. Thus, the objectives of the present studies were to ascertain the extent of bioavailability of soil-bound residues of DDT and HCH to earthworm, *Pheretima posthuma* Vaillant, as earthworms are known to easily accumulate pesticide residues and exhibit high levels of biomagnification[11].

As radionuclear techniques are essential for quantification of bound residues, uniformly ring labelled (^{14}C)-*p,p'*-DDT (specific activity 3.14 GBq mM^{-1}) and γ-(^{14}C)-HCH (specific activity 2 GBq mM^{-1}) obtained from Radiochemical Centre, Amersham International, England, were employed. A fallow field with sandy-loam-type soil having 0.8% organic carbon was selected for the study. Soil-bound residues of DDT or HCH were generated by surface application of ^{14}C-labelled insecticidal solutions, and after one year the soil samples were soxhlet extracted with methanol as described by Samuel *et al.*[10] Further extraction of soil with acetone–hexane mixture did not yield any extractable residues. To estimate the bound residues, 300 mg samples of extracted soil was dried and subjected to combustion in a Harvey's Biological

Material Oxidizer (OX–400) to $^{14}CO_2$. The $^{14}CO_2$ trapped in carbon-14 cocktail was directly used for radioassay. A minimum of four replicates were used for extraction and combustion. The following soil types, as indicated in Tables 1 and 2, were used for the study.

Soil I: One-year-old treated soil, containing only bound residues of DDT or HCH.

Soil II: One-year-old treated soil, containing both extractable and bound residues of DDT or HCH.

Soil III: Soil, freshly treated with DDT or HCH and having residues predominantly in the extractable form.

The different soil samples (each weighing 800 g) moistened to 75% field capacity were put in polythene packets (36 × 23 cm) and after 24 h, 15 healthy laboratory-acclimatized earthworms were introduced in each packet. The worms were maintained under controlled conditions at 27 ± 2°C for 28 days and for each soil type three replicates were used. The earthworms were fed by providing 1 g cornflour in 10 ml water twice a week on the soil surface. From each packet, three worms sampled at weekly intervals were rinsed thoroughly in water to remove adhering soil and introduced in petri dishes lined by moist filter papers for 42 h to allow their gut soil to be void. The purged worms were washed, soaked in filter-papers, weighed and stored at −20°C. Soil samples were taken prior to the introduction of the earthworms and on completion of the experiments. As control, separate samples of soil I were kept under identical conditions without earthworms to assess the release of bound residues. The earthworms were extracted with hexane : acetone (1 : 1)[11] and subsequently radioassayed to ascertain the extractable residue fraction in the animals. The bound residue fraction was analysed by subjecting the extracted samples to combustion in the biological oxidizer.

An Aloka liquid scintillation spectrometer was used for radioassay. Bray's cocktail was employed for methanolic samples (soil), while a toluene-based cocktail containing PPO (5 g l^{-1}) and POPOP (50 mg l^{-1}) was used for hexane samples (earthworms). The efficiency of analytical procedures was estimated by spiking known amounts of (^{14}C)-DDT and HCH into various samples. Thus, soil extraction gave 96 to 98.5% and combustion 97.5% recovery for both DDT and HCH. The extraction efficiency of DDT and HCH from earthworms varied from 91.8 to 94.1%, while that of combustion was 97.1%. All the data presented are corrected for background counts and the efficiency of analytical procedures.

Earthworms exposed in soil I containing only bound residues of DDT picked up 0.05% (g^{-1} wet weight basis) of the total residues present in the soil in seven days and the rate of accumulation remained steady for the rest 28 days (Figure 1). Out of the total residues, 40% was found to be tissue-bound till day 14, though the worms sampled subsequently did not possess any tissue-bound DDT residues (Figure 1). Under identical conditions earthworms from soil II picked up 0.27 to

Table 1. Total amount of [^{14}C]-DDT residues (10^3 dpm/800 g ± SE) present in different soil types.

Soil type	Initial [^{14}C]-DDT residues (0 day)			Final [^{14}C]-DDT residues (after 28 days)		
	Extractable	Bound	Total	Extractable	Bound	Total
With earthworm						
I	ND	652 ± 24	652 ± 24	99 ± 5	524 ± 10	623 ± 5
II	5062 ± 70	913 ± 53	5975 ± 18	4395 ± 26	609 ± 8	5004 ± 17
III	645 ± 16	47 ± 6	692 ± 11	372 ± 27	51 ± 5	423 ± 22
Without earthworm						
I	ND	652 ± 24	652 ± 24	23 ± 2	613 ± 10	636 ± 11

ND, Not detected.

Table 2. Total amount of γ-[^{14}C]-HCH residues (10^3 dpm/800 g ± SE) present in different soil types.

Soil type	Initial γ-[^{14}C]-HCH residues (0 day)			Final γ-[^{14}C]-HCH residues (after 28 days)		
	Extractable	Bound	Total	Extractable	Bound	Total
With earthworm						
I	ND	979 ± 13	979 ± 13	202 ± 7	716 ± 11	918 ± 5
II	1186 ± 31	1391 ± 12	2577 ± 19	1195 ± 2	1057 ± 7	2252 ± 9
III	754 ± 66	122 ± 11	876 ± 59	34 ± 2	314 ± 2	348 ± 2
Without earthworm						
I	ND	979 ± 13	979 ± 13	39 ± 3	921 ± 35	960 ± 37

ND, Not detected.

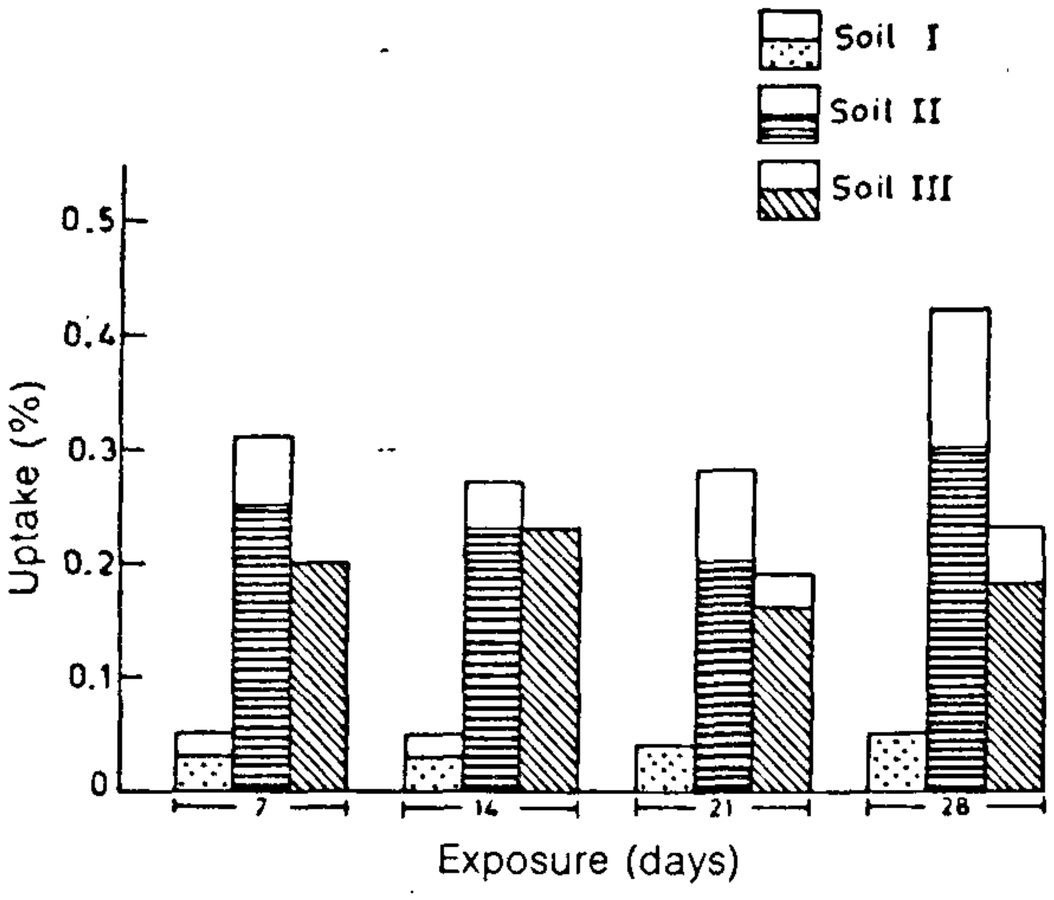

Figure 1. Uptake of [^{14}C]-DDT by earthworms as per cent of initial concentration of DDT residues in soil on g^{-1} wet weight basis. Histograms indicate per cent distribution of DDT residues in the extractable (shaded) and bound (blank) fractions.

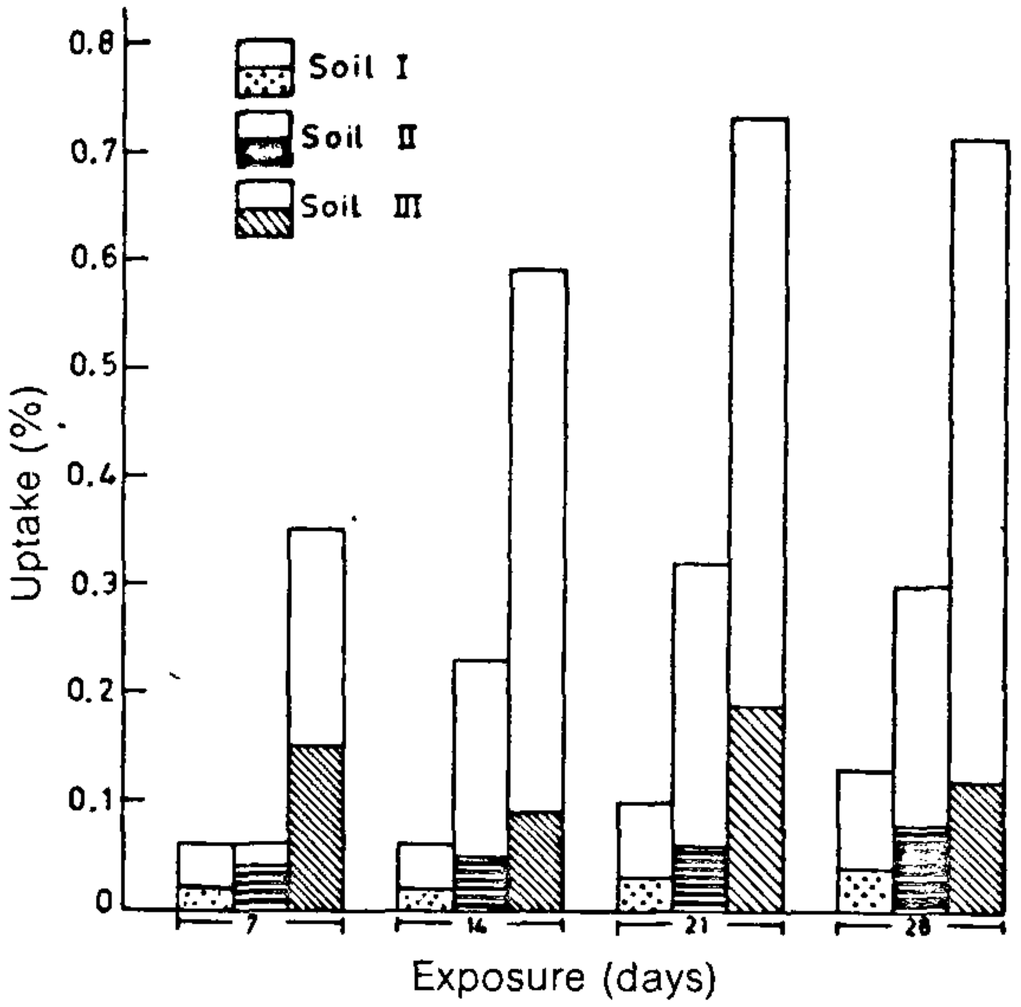

Figure 2. Uptake of γ-[^{14}C]-HCH residues by earthworms as per cent of initial concentration of HCH residues in soil on g^{-1} wet weight basis. Histograms indicate per cent distribution of HCH residues in the extractable (shaded) and bound (blank) fractions.

0.42% of DDT residues, while from soil III the bioaccumulation ranged from 0.19 to 0.23%. The rate of uptake of DDT from soil II and soil III was 8.4 and 4.0 times higher compared to soil I. The earthworms from soil II contained 15 to 28% of tissue-bound residues of DDT in the course of 28 days while in the case of earthworms collected from soil III no tissue-bound residues were detected for 14 days, but from 21 days tissue-bound fraction varied from 16 to 22%. Thus, the bound residues show a reverse trend in soil I and soil III.

When earthworms were exposed to soil I containing only bound residues of HCH, the rate of bioaccumulation steadily increased from 0.06 to 0.13% within 28 days while in the case of uptake from soil II the residues increased from 0.06 to 0.32% (Figure 2). Maximum bioconcentration was evident in earthworms from soil III as it ranged from 0.35 to 0.73% of the total HCH residues present in the soil. Earthworms from all the soil samples contained residues predominantly in the bound form varying from 70 to 84% (Figure 2).

The overall uptake of DDT was more from soil II unlike in the case of HCH where maximum uptake was evident from soil III. This is evident due to the fact that soil II had more of labile DDT in relation to bound residues unlike in the case of HCH where soil II contained more than 60% residues in bound form. Also, proportionately more labile HCH was available in soil III compared to soil II which enhanced the chances of bioaccumulation in earthworms. Tissue-binding of HCH and DDT in earthworms, as evident from the present data, indicates differential behaviour of HCH and DDT. HCH excelled DDT in tissue binding in all the instances, thus registering a maximum of 87% binding from soil III compared to 28% of DDT from soil II. It is well documented that HCH has higher binding capacity than DDT[10].

The soil samples after 28 days of experiment showed reduction in the radioactivity levels (Tables 1 and 2). Thus, soil I recorded 5 to 6% loss while soil II had 13 to 16% loss of DDT and HCH residues (Tables 1 and 2). Maximum loss of residues in 28 days was observed in soil III accounting 40% of DDT and 60% of HCH. Major loss in soil residues may be largely due to volatilization of DDT and HCH as reported by Samuel *et al.*[10] and the loss was more when the residues were in the labile form. It is evident from the data that 15% DDT and 20% HCH of soil-bound residues from soil I became mobilized to extractable form within 28 days, while soil I without earthworms showed only 4% mobilization (Tables 1 and 2). This indicates that the earthworms facilitate faster release of bound residues of DDT and HCH as large quantities of soil pass through the gut and get exposed to many digestive enzymes.

The present data clearly indicate that the earthworms are capable of bioaccumulating a low concentration of soil-bound DDT and more of soil-bound HCH residues. Similarly, Fuhremann and Lichtenstein[6] reported sizable amounts of ^{14}C in *Lumbricus terrestris* when exposed to soil-bound residues of (^{14}C)-methyl parathion for six weeks, and 58 to 66% of the accumulated residues were tissue-bound.

Two major conclusions emerge from the present studies. Soil-bound residues of DDT and HCH are bioavailable to earthworms. Also, earthworms facilitate

mobilization of soil-bound residues, indicating the potential toxicological hazards of soil-bound residues of DDT and HCH.

1. Kaufman, D. D., Still, G. G., Paulson, G. D. and Bandal, S. K. (eds.), *Bound and Conjugated Pesticide Residues*, Amersham Chemical Society Symposium Series 1976, No. 29, p. 396.
2. Lichtenstein, E. P., *Residue Rev.*, 1980, **76**, 147.
3. Khan, S. U., *Residue Rev.*, 1982, **84**, 1.
4. Khan, S. U. and Ivarson, K. C., *J. Agric. Food Chem.*, 1981, **29**, 1301.
5. Racke, K. D. and Lichtenstein, E. P., *J. Agric. Food Chem.*, 1985, **33**, 938.
6. Fuhremann, T. W. and Lichtenstein, E. P., *J. Agric. Food Chem.*, 1978, **26**, 605.
7. Khan S. U., *J. Agric. Food Chem.*, 1980, **28**, 1096.
8. Haque, A., Schupan, I. and Ebwing, W., *Pestic. Sci.*, 1982, **13**, 219.
9. Khan, S. U., Kacew, S., Dupont, S., Stratton, G. D., Jr. and Wheeler, W. B., *J. Agric. Food Chem.*, 1987, **35**, 89.
10. Samuel, T., Agarwal, H. C. and Pillai, M. K. K., *Pestic. Sci.*, 1988, **22**, 1.
11. Yadav, D. V., Pillai, M. K. K. and Agarwal, H. C., *Bull. Environ. Contam. Toxicol.*, 1976, **16**, 541.

ACKNOWLEDGEMENTS. Award of a fellowship to AV from the University Grants Commission, New Delhi, is gratefully acknowledged.

Received 15 October 1990; revised accepted 4 May 1991