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Incorporation of Methoxychlor-^{14}C in Model Humic Acids Prepared from Hydroquinone*

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The inordinate retention of pesticides in soils (CHIBA and MORLEY 1968a; MORLEY 1972; MATHUR and MORLEY 1975; STEVENSON 1976), particularly in organic soils (MATHUR et al 1976a; 1976b; MATHUR and SAHA 1977), suggests that, in addition to the fraction that is adsorbed (MORITA 1976), a portion of certain pesticides may be incorporated or 'locked' into soil humus. The examination of this possibility through extraction and chemical degradation of humic materials treated with pesticides in the field is difficult, due to the nature of soil humus (MATHUR 1971). Also, the possibilities of biodegradation of the pesticide render the use of isotope-labelled materials in the field ineffective for such studies. One alternative is to use biodegradation to resolve complexes of pesticides and humic materials into identifiable units. For this, the humates used may be natural (HSU and BARTHA 1974) or models synthesized biologically or chemically. Analogues offer the opportunity to study incorporation of pesticides during synthesis, rather than their reactions only with the relatively stable humus.

The use of model humic acids has contributed considerably towards our understnading of the chemical structure and behavior of soil humus (LADD and BUTLER 1966; MARTIN and HAIDER 1971; PIPER and POSNER 1972; ROY et al 1973; ROWELL et al 1973; HAIDER and MARTIN 1976; NELSON et al 1976). Consequently, the use of humus analogues for investigating pesticide behavior in soils was attempted by MATHUR and MORLEY (1975). They found that the insecticide methoxychlor present in the culture of a fungus was incorporated at the rate of about 668 ppm into a humic acid-like pigment produced by this organism. This indication of the possibility of incorporation of this pesticide into soil humus, and of the feasibility of using model humic acids for such studies was further investigated here by having ^{14}C-labelled methoxychlor present during the chemical synthesis of a humic acid analogue.

MATERIALS AND METHODS

Recrystallized, pure (99%+) 2,2-bis (p-methoxyphenyl)1,1,1- trichloroethane (methoxychlor) was obtained from Canadian Industries Ltd.; universally ring-labelled methoxychlor-^{14}C from Mallinckrodt Nuclear, St. Louis, Mo.; and hydroquinone (p-dihydroxybenzene) from Sigma Chemical Co. All solvents used were pesticide-grade and used as received.

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Preparation of humic acids

The procedure of CORNEC and BRUSSET (1950) was adapted as follows to prepare humic acid (HA) analogues. Hydroquinone (3 g) was dissolved in 250 ml of 0.1 M pH 9.6, NH_4OH - NH_4Cl buffer in a 500 ml glass-stoppered Erlenmeyer flask. To this, 100 mg of $FeSO_4.H_2O$, 3 ml of ethanol containing 5 mg ^{14}C-labelled methoxychlor (specific activity $\approx$ 3.80 mCi/mM), and 2.5 g of sodium persulfate were added. The contents were mixed thoroughly and stored for 18 hours in the dark at 21°C. The contents were then transferred into a dialysis tube and dialysed against distilled water until free of Cl^- and SO_4^{--} ions. The dialysed suspension was concentrated in a rotary evaporator at 35°C and freeze-dried. As controls, (a) the HA analogue check was prepared in the absence of the insecticide, and (b) methoxychlor was subjected to the same procedure in the absence of hydroquinone.

Analyses

Mineral, carbon and hydrogen contents of the HA analogues were determined by the Pregl method through combustion in dry air in a Sargent furnace; nitrogen by the automated micro-Dumas method using a Coleman model 29 N-analyzer; and sulfur by oxygen-flask combustion. Total acidity and carboxyl groups of the humic acid analogues were measured by barium hydroxide and calcium acetate methods as described by SCHNITZER (1972). IR and ESR spectra were also recorded (SCHNITZER 1971; MATHUR 1972).

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Extractions

A sample of the control HA analogue prepared in the absence of methoxychlor was wetted with an ethanol solution of methoxychlor in a glass fiber thimble and allowed to dry. The HA analogue prepared in the presence of methoxychlor was also placed in a glass fiber thimble and the two thimbles extracted separately with acetone/hexane (1:1) in a Goldfisch apparatus for various durations. In another set up, the control HA analogue was similarly extracted to provide background data.

Estimation of methoxychlor

All extracts were transferred to hexane and their radioactivity counted as described by CHIBA and MORLEY (1968a) using a toluene solution of 0.5%, 2,5-diphenyloxazole (PPO) and 0.05% p-bis, 2-(5-phenyloxazoyl) benzene (Nuclear Chicago Corp., Des Plaines, Ill.) and a Liquid Scintillation Spectrophotometer (Nuclear Chicago Model 703). These solutions were also subjected to quantitative gas chromatography as described by MATHUR and MORLEY (1975) to determine the amount of methoxychlor they contained.

The radioactivity contained in the control HA analogue and the one synthesized in the presence of methoxychlor was determined, both before and after exhaustive extractions, by counting the $^{14}CO_2$ produced by their dry combustion (Packard Instruments), and absorbed in 1M Hyamine hydroxide.

RESULTS AND DISCUSSION

The yields of the HA analogues synthesized in the absence or presence of the chlorinated hydrocarbon insecticide were 948 and 987 mg, respectively.

TABLE I

Elemental and functional group analyses of the humic acid analogues (HA) on dry mineral-free basis

Property	HA synthesized in the absence of methoxychlor	HA synthesized in the presence of methoxychlor
Mineral matter %	1.23	1.97
Carbon %	58.34	56.39
Hydrogen %	4.65	4.42
Nitrogen %	4.06	3.45
Sulfur %	0.12	0.13
Oxygen*%	32.83	35.61
Total Acidity meq/g	5.05	5.15
Carboxyl groups meq/g	2.32	2.82
Phenolic hythroxyl* groups meq/g	2.73	2.33

* by difference

Data presented in Table I show that the HA analogues were similar to soil humic acids as described in these respects by SCHNITZER (1972) and SCHNITZER and KHAN (1972). Also IR and ESR spectra of these analogues (SCHNITZER and MATHUR 1977) resembled those obtained for soil humic compounds (SCHNITZER 1971; MATHUR 1972). Further, both analogues were found by enrichment culture techniques (MATHUR 1969) to be resistant to microbial degradation, again like soil humates.

All of the radioactivity added as methoxychlor to the buffer while the hydroquinone was being polymerized was found to be present in the HA analogue produced. In the absence of the hydroquinone, the radioactivity was totally recoverable by a single extraction of the reaction medium with acetone:hexane. Quantitative assay of this extract revealed that the radioactivity was entirely due to methoxychlor, thus showing that the insecticide was stable under the conditions employed for polymerization of the hydroquinone. Similarly, the methoxychlor added to the control

HA analogue (synthesized in absence of the insecticide) was also completely (99%+) recovered by a single 18 hr extraction with acetone:hexane, indicating that the polymer did not interfere with solvation and extraction of the methoxychlor. Identical extraction of the HA analogue synthesized in the presence of the insecticide, however, yielded only 91.59 percent of the radioactivity present in the sample. Second, third and fourth extractions for 6, 16 and 18 hrs. yielded further 2.32, 0.45 and 0.41 percents, respectively of the total radioactivity in the sample. Quantitative estimations by gas chromatography showed that the methoxychlor contents of these extracts accounted for all of the radioactivity they contained. Further extractions with acetone:hexane or methanol did not yield measurable amounts of radioactivity or methoxychlor.

The residue of the fourth extraction of the same HA analogue was found to contain, on combustion, 5.1 percent of the radioactivity originally present in the sample, thus accounting for nearly all of the activity not extracted. This activity retained in the analogue could have been due to (a) methoxychlor exclusively or include (b) HA structures derived from the insecticide. If entirely due to (a), it corresponded to 263 ppm of methoxychlor in the exhaustively extracted material. On two complete repetitions of the above experiments, the corresponding value was 248 ppm the first time and 200 ppm the second time, again representing retention of about 4 to 5 percent of the total methoxychlor present. It should be emphasized that the level of methoxychlor present in the original reaction medium was only 20 ppm with respect to the solution in which the HA was synthesized, about 1700 ppm with respect to the hydroquinone, and nearly 5000 ppm of the final product. These values are comparable to the concentrations which may occur in microsites in the field where granular pesticide formulations are sometimes applied in bands (e.g. see MATHUR et al, 1976a).

The suggestions made earlier (MATHUR and MORLEY 1975) that pesticides may be incorporated into soil humic materials by being structurally bound or held by forces stronger than those of conventional physical adsorption, and that this process may be investigated through model humic acids, are thus strengthened. This study also indicated that the persistence and mobility of the biodegradable and water-insoluble methoxychlor, as of other pesticides (MORLEY 1972), may be affected by its incorporation into newly synthesized humic materials some of which are water soluble. Hundreds of Kg of such humic materials are synthesized per ha even in mineral soils every year, and the time of contact between added chemicals and soil organic matter is usually longer *in situ* than the 18 hours in this laboratory study.

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LITERATURE CITED

CHIBA, M. and H.V. MORLEY: J. Agr. Food Chem. 16. 916 (1968a).
CHIBA, M. and H.V. MORLEY: J. Assoc. Offic. Anal. Chemists 51. 55 (1968b).
CORNEC, P. and H. BRUSSET: C.R. Acad. Sci. Paris 230. 1890 (1950)
HAIDER, K. and J.P. MARTIN: Agron. Abstt. Amer. Soc. Agron. p. 136. (1976).
HSU, T-S., and R. BARTHA: Soil Sci. 118. 213 (1974).
LADD, J.N. and J.H.A. BUTLER: Aust. J. Soil Res. 4. 41 (1966).
MARTIN, J.P. and K. HAIDER: Soil Sci. 111. 54 (1971).
MATHUR, S.P.: Can. J. Microb. 15. 677 (1969).
MATHUR, S.P.: Soil Sci. 111. 147 (1971).
MATHUR, S.P.: Soil Sci. Soc. Amer. Proc. 36. 611 (1972).
MATHUR, S.P.; A. BELANGER; S.U. KHAN; H.A. HAMILTON; R. GREENHALGH and K.A. MACMILLAN: Weed Res. 16. 183 (1976a).
MATHUR, S.P.; H.A. HAMILTON; R. GREENHALGH; K.A. MACMILLAN and S.U. KHAN: Canad. Jour. Soil Sci. 56. 89 (1976b).
MATHUR, S.P. and H.V. MORLEY: Soil Sci. 120. 238 (1975).
MATHUR, S.P. and J.G. SAHA: Bull. Environ. Contam. & Toxicol. 17. 424 (1977).
MORITA, H.: Can. J. Soil Sci. 56. 105 (1976).
MORLEY, H.V.: Soil-pesticide interactions. p. 69 in Intern. Synposium on Identification and Measurement of Environ. Pollutants, Ottawa. June 1971. National Research Council of Canada (1972).
NELSON, D.W.; J.P. MARTIN and J.O. ERVIN: Agron. Abstt. Amer. Soc. of Agron. p. 137 (1976).
PIPER, T.J. and A.M. POSNER: Soil Biol. and Biochem. 4. 513 (1972).
ROWELL, M.J.; J.N. LADD and E.A. PAUL: Soil Biol. and Biochem. 5. 699 (1973).
ROY, K.B.; S.K. BANNERJEE and S.K. MUKHERJEE: J. Indian Chem. Soc. 50. 401 (1973).
SCHNITZER, M.: Characterization of humic constituents by spectroscopy. in A.D. McLaren and J. Skujins (Eds.) Soil Biochemistry 2, Marcel Dekker Inc. New York. p. 60-95 (1971).
SCHNITZER, M.: p. 293. Proc. Int. Meet. Humic Substances. Nieuwersluis, Pudoc, Wageningen. (1972).
SCHNITZER, M. and S.U. KHAN: Humic substances in the environment. Marcel Dekker Inc., New York (1972).
SCHNITZER, M. and S.P. MATHUR: unpublished data (1977)
STEVENSON, F.J.: Organic matter reactions involving pesticides in soil. in Bound and Conjugated Pesticide Residues. Amer. Chem. Soc. Symp. series no. 29. 180 (1976).

LITERATURE CITED

CHIBA, M. and H.V. MORLEY: J. Agr. Food Chem. 16. 916 (1968a).

CHIBA, M. and H.V. MORLEY: J. Assoc. Offic. Anal. Chemists 51. 55 (1968b).

CORNEC, P. and H. BRUSSET: C.R. Acad. Sci. Paris 230. 1890 (1950).

HAIDER, K. and J.P. MARTIN: Agron. Abstt. Amer. Soc. Agron. p. 136. (1976).

HSU, T-S., and R. BARTHA: Soil Sci. 118. 213 (1974).

LADD, J.N. and J.H.A. BUTLER: Aust. J. Soil Res. 4. 41 (1966).

MARTIN, J.P. and K. HAIDER: Soil Sci. 111. 54 (1971).

MATHUR, S.P.: Can. J. Microb. 15. 677 (1969).

MATHUR, S.P.: Soil Sci. 111. 147 (1971).

MATHUR, S.P.: Soil Sci. Soc. Amer. Proc. 36. 611 (1972).

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MATHUR, S.P.; H.A. HAMILTON; R. GREENHALGH; K.A. MACMILLAN and S.U. KHAN: Canad. Jour. Soil Sci. 56. 89 (1976b).

MATHUR, S.P. and H.V. MORLEY: Soil Sci. 120. 238 (1975).

MATHUR, S.P. and J.G. SAHA: Bull. Environ. Contam. & Toxicol. 17. 424 (1977).

MORITA, H.: Can. J. Soil Sci. 56. 105 (1976).

MORLEY, H.V.: Soil-pesticide interactions. p. 69 in Intern. Symposium on Identification and Measurement of Environmental Pollutants, Ottawa. June 1971. National Research Council of Canada (1972).

NELSON, D.W.; J.P. MARTIN and J.O. ERVIN: Agron. Abstt. Amer. Soc. of Agron. p. 137 (1976).

PIPER, T.J. and A.M. POSNER: Soil Biol. and Biochem. 4.513 (1972).

ROWELL, M.J.; J.N. LADD and E.A. PAUL: Soil Biol. and Biochem. 5. 699 (1973).

ROY, K.B.; S.K. BANNERJEE and S.K. MUKHERJEE: J. Indian Chem. Soc. 50. 401 (1973).

SCHNITZER, M.: Characterization of humic constituents by spectroscopy. in A.D. McLaren and J. Skujins (Eds.) Soil Biochemistry 2, Marcel Dekker Inc. New York. p. 60-95 (1971).

SCHNITZER, M.: p. 293. Proc. Int. Meet. Humic Substances. Nieuwersluis, Pudoc, Wageningen. (1972).

SCHNITZER, M. and S.U. KHAN: Humic substances in the environment. Marcel Dekker Inc., New York (1972).

SCHNITZER, M. and S.P. MATHUR: unpublished data (1977).

STEVENSON, F.J.: Organic matter reactions involving pesticides in soil in Bound and Conjugated Pesticide Residues. Amer. Chem. Soc. Symp. series no. 29. 180 (1976).