New Interlamellar Pesticide–Metal–Montmorillonite Complexes: A Novel Technique for Controlled Release[1]

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Encapsulation of pesticides via complexation with metals in the interlamellars of montmorillonite, a smectite clay, as a technique for controlled release, a potential alternative to the immobilization of pesticides in polymers, has been presented for the first time. Diazinon, chlorpyrifos, carboxin, and carbendazim have been chelated with Cu/Co in the interlamellars of montmorillonite as evidenced by analytical, IR, XRD, and ESR data. Chemical assay results indicate that the intercalated pesticides were released in small amounts over a period of 7–8 weeks. On the other hand, adsorbed pesticides were totally leached within 24 h from montmorillonite. The general and persistent toxicity tests for these complexes against housefly (*Musca domestica*) demonstrate that the diazinon and chlorpyrifos clay complexes showed 100% mortality for an extended period of 20–22 weeks against analogous conventional formulations exhibiting 100% mortality up to 4–8 weeks only. These results, which suggest prolonged bioactivity in agriculture, propose the present encapsulation technique for controlled release as a potential alternative to the well-established immobilization methods.

Recent interest has been focused on the intercalation of various substrates in montmorillonite as a technique for novel applications such as model enzyme immobilization (Kijima et al., 1984), heterogenization of transition-metal catalysts (Pinnavaia, 1983; Choudary and Bharathi, 1987), and detoxification of pesticides (Boyd and Mortland, 1985; Mortland and Raman, 1967). Polymerization of dioxin and hydrolysis of organic phosphate insecticides are the processes employed for detoxification. The diversity in the use of montmorillonite has been further demonstrated with the report of photochemical stability of insecticidal compounds upon intercalation in the montmorillonite (Margulies et al., 1988). The interaction of phenamiphos with various metals present in the montmorillonite has been described very recently (Rodriguez et al., 1988).

Controlled release of pesticides has been widely explored because it can have the advantage of enhanced activity for a prolonged time with reduced content of active ingredient when compared with conventional formulations. Immobilization of the pesticides with the polymers by chemical and physical methods is well-known (Das, 1983). The present new technique for controlled release of pesticides, reported in this paper, involves the encapsulation of pesticides via complexation with metal in the interlamellars of smectite clay. We chose montmorillonite a naturally abundant and cheaply available smectite clay for encapsulation of pesticides, since presently used synthetic polymers, like polyethylene, poly(vinyl chloride), poly(acryloyl chloride), etc., for encapsulation of pesticides are expensive and nonbiodegradable, contaminating the soil. The present technique has potential advantages including enhancement of the persistence and reduction of mammalian toxicity.

In the present paper, we report synthesis, characterization, and bioassay of new pesticide–metal–montmorillonite complexes, diazinon–Co(II)–montmorillonite (1), diazinon–Cu(II)–montmorillonite (2), chlorpyrifos–Co(II)–montmorillonite (3), chlorpyrifos–Cu(II)–montmorillonite (4), carboxin–Co(II)–montmorillonite (5), carboxin–Cu(II)–montmorillonite (6), carbendazim–Co(II)–montmorillonite (7), and carbendazim–Cu(II)–

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montmorillonite (8), and the release rate patterns of diazinon [*O,O*-diethyl *O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate], chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate], carboxin [5,6-dihydro-2-methyl-*N*-phenyl-1,4-oxathiin-3-carboxamide], and carbendazim [methyl 1*H*-benzimidazol-2-yl-carbamate] from the clay complexes.

MATERIALS AND METHODS

Safety precautions: Direct skin contact is avoided. All experiments should be conducted in hood.

Materials. Montmorillonite was purchased from Fluka Chemie AG, CH-9470 Buchs. Cu(II)–montmorillonite was prepared as described by Kijima et al. (1984). Freshly prepared diazinon, chlorpyrifos, carboxin, and carbendazim were procured from the Pesticide Division, Regional Research Laboratory, Hyderabad, India.

IR and FT-IR spectra were recorded on Perkin-Elmer 283-B and Digilab FTS-14, respectively. XRD patterns were recorded on Phillips, PW-1057 diffractometer with Ni-filtered Cu Kα radiation (1.541 38 Å). TGA was carried out with the equipment designed and fabricated by the Regional Research Laboratory, Hyderabad. ESR spectra were recorded on a Bruker ER-200D. Standard methods were used for determining the elements (excluding the analysis of montmorillonite). The GC (Hitachi 063) was used for quantitative estimation of the pesticides in the release rate determination. The following GC conditions were employed for the estimation of diazinon, chlorpyrifos, and carboxin: column, 240 cm × 3 mm (i.d.), stainless steel tubes; packing, 10% OV-17 on 60–80-mesh Chromosorb WAW; carrier gas, nitrogen at 60 mL/min; detector, FPD; column temperature, 150–260 °C; injection temperature, 300 °C; detector temperature, 350 °C; recorder range, 1 mV; chart speed, 5 mm/min; attenuation, 8. Carbendazim was estimated as its derivative (Tjan and Jansen, 1979) by GC equipped with the electron capture detector and a borosilicate glass column, 160 cm × 3 mm (i.d.), with 3% OV-1 packing on 80–100-mesh Chromosorb W (HP). Other conditions: carrier gas, argon–methane (95:5) at 60 mL/min; column temperature, 240 °C; detector temperature, 350 °C; injection temperature 250 °C; attenuation, 64; chart speed, 5 mm/min.

The test insects, *Musca domestica* (housefly), were reared under controlled temperature (27 ± 1 °C) and humidity conditions (65–75%) in the insectary.

Preparation of the Complexes. Pesticide–metal(II)–montmorillonite complexes were prepared by refluxing 1 g of metal(II)–montmorillonite (Co, 6.4%; Cu, 6.0%) and the ligand (metal to ligand molar ratio is 1:3) in 100 mL of methanol for 6 h with constant stirring. The reaction mixture was filtered, washed with petroleum ether (60–80 °C; 3 × 10 mL) and ether (3 × 10 mL), and dried under vacuum for all the pesticide complexes

Pesticide–Metal–Montmorillonite Complexes

J. Agric. Food Chem., Vol. 37, No. 5, 1989 **1423**

Table I. Analytical Data for Pesticide–Metal(II)–Montmorillonite Complexes (Clay Complexes)

clay complex	elemental analysis,[a] % found (required)					
	Co[b]	Cu[b]	P	S	N	Cl
1	6.40		2.34	2.43	2.12	
			(3.36)	(3.48)	(3.04)	
2		6.00	2.04	2.12	1.85	
			(2.92)	(3.03)	(2.60)	
3	6.40		2.24	2.32	1.01	7.70
			(3.36)	(3.48)	(1.52)	(11.57)
4		6.00	2.01	2.09	0.91	6.93
			(2.92)	(3.03)	(1.32)	(10.06)
5	6.40			2.76	1.21	
				(3.48)	(1.52)	
6		6.00		2.35	1.02	
				(3.03)	(1.32)	
7	6.40				3.59	
					(4.56)	
8		6.00			3.25	
					(3.97)	

[a] Analysis indicates percent ratio of metal to heteroatoms of the ligands only. The difference from permissible was due to noncomplexation of some of the exchanged metal. [b] The amount of the metal found is taken as standard for theoretical calculations too.

except the carbendazim complexes, which were washed with hot ethanol and dried under vacuum. Montmorillonite was suspended in an ethanolic solution of the pesticide for 24 h at room temperature. The solution was decanted, and adsorbed pesticide onto montmorillonite was dried.

Chemical Assay. Two hundred milligrams of the freshly prepared clay complex was placed in 100 mL of distilled water (triplicate for each week) in an iodometric flask to minimize loss of water by evaporation and then allowed to stand at room temperature. Flasks were shaken once a week and before each sampling. At the end of each week the pesticide (diazinon, chlorpyrifos, carboxin) released in each flask was extracted with dichloromethane. The extract was reduced to 5 mL and then made up to 10 mL in a standard flask with dichloromethane. The extracts were analyzed to determine the amount of pesticide released by GC based on the calibration curve obtained from standard samples. The release rates of carbendazim–clay complexes were determined by GC after extraction with ethyl acetate and preparing its derivative with pentafluorobenzyl bromide and isooctane (Tjan and Jansen, 1979). Similarly, the samples of pesticides adsorbed onto montmorillonite were subjected to same treatment as described, but samples were drawn hourly for analysis.

General and Persistent Toxicity. The pesticides (technical grade unformulated) and their respective clay complexes were individually diluted with sucrose to obtain a concentration of 0.05% of active agent. The containers with mixed powders were placed inside 1-L beakers and kept ready to receive the test insects. Each beaker received 20 5-day-old male insects. Mortality in each experiment was recorded 24 h posttreatment. Each individual experiment was conducted in triplicate, and the mean mortality was recorded. A control experiment with sucrose alone was also run to record the natural mortality. The beakers were left undisturbed after the flies were removed and every week a fresh batch of 20 5-day-old male flies were released. The experiment continued on a weekly basis until the experimental compounds stopped giving appreciable mortality.

RESULTS AND DISCUSSION

Characterization of Pesticide–Metal–Montmorillonite Complexes. Elemental analysis data indicated the exchanged metal(II)–pesticide ratio in complexes 1 (1:0.8), 2 (1:0.7), 3 (1:0.7), 4 (1:0.7), 5 (1:0.86); 6 (1:0.7), 7 (1:0.85), and 8 (1:0.77) to be approximately 1:1 (Table I). The difference than permissible was due to noncomplexation of some of the exchanged metal.

The thermogravimetric analyses (TGA) of all the clay complexes exhibit the loss in weight in two steps. The first step takes place between 50 and 130 °C and was attributed

Table II. IR and FT IR Spectral Data of Pesticide–Metal(II)–Montmorillonite Complexes (Clay Complexes)

	clay complex[a]		band assgnt
ligand	Co(II)	Cu(II)	
	Diazinon		
	200 m	185 m	ν(M—S)
	260 m	260 m	ν(M—N)
1560 s	1590 m	1590 m	ν(C=N)
	Chlorpyrifos		
	230 m	230 m	ν(M—S)
	255 m	265 m	ν(M—N)
1540 s	1545 m	1545 m	ν(C=N)
	Carboxin		
	235 w	235 w	ν(M—S)
	420 sh	420 w	ν(M—O)
1645 s	1590 w	1600 w	ν(C=O)
	Carbendazim		
	260 w	265 w	ν(M—N)
	435 w	435 w	ν(M—O)
1630 s	1650 s	1640 s	ν(C=O)
1440 s	1445 s	1450 s	ν(C=N)

[a] Key: s = strong; m = medium; sh = shoulder; w = weak.

Table III. XRD Spacing for Pesticide–Metal(II)–Montmorillonite Complexes (Clay Complexes)

clay complex	2θ value	d_{001} spacing, Å	clay complex	2θ value	d_{001} spacing, Å
1	5.3	16.674	5	5.4	16.365
2	5.2	16.994	6	5.36	16.488
3	5.1	17.327	7	5.2	16.994
4	5.16	17.126	8	5.2	16.994

Table IV. ESR Data of Pesticide–Cu(II)–Montmorillonite Complexes

complex	$g_{\parallel}$	$g_{\perp}$	g_1	g_2	g_3	g_{av}
2	1.366	1.484				1.405
4	1.366	1.489				1.407
6			2.29	2.15	2.03	2.16
8			2.302	2.12	2.05	2.15

to the loss of water molecules. No loss in weight was observed for complexes 1–6 in the range 130–180 °C and in complexes 7 and 8 in the range 130–250 °C. This indicates the stability of the complexes in that temperature range. The total decomposition of the anhydrous pesticide–clay complexes taking place at 650 °C was shown by the loss of pesticide moiety. The results agreed very closely with the elemental analysis data (metal:pesticide ≈ 1).

Table II describes IR and FT IR data for clay complexes 1–8. The sharp bands at 1560 cm⁻¹ assigned to ν(C=N) in diazinon (Giang, 1977), at 1540 cm⁻¹ in chlorpyrifos (van der Marel and Beutelspacher, 1976), and 1440 cm⁻¹ in carbendazim were considerably shifted to lower wavelengths (1590, 1545, and 1445 cm⁻¹, respectively) in complexes 1–4, 7, and 8, indicating N-coordination to the metal. The bands assigned to ν(C=O) at 1645 cm⁻¹ in carboxin (Giang, 1977) and at 1630 cm⁻¹ in carbendazim were considerably shifted in complexes 5–8, indicating coordination through the O atom. The ν(P=S) bands in clay complexes 1–4 were not observed due to the interference of montmorillonite.

In addition to the characteristic montmorillonite absorption peaks (van der Marel and Beutelspacher, 1976) clay complexes 1–8 exhibit two bands in the FT IR spectra. No such bands were observed in the spectra of the free ligand. The band in the region 185–235 cm⁻¹ appearing in clay complexes 1–6 was assigned to ν(M–S) and the band at 250–265 cm⁻¹ in complexes 1–4, 7, and 8 was assigned to ν(M–N) (Ferraro, 1971). The ν(M–O) band appeared

1424 *J. Agric. Food Chem.*, Vol. 37, No. 5, 1989

Choudary et al.

Table V. Release Rate Pattern of Pesticide-Metal(II)-Montmorillonite Complexes

complex	% release of pesticide/week							
	I	II	III	IV	V	VI	VII	VIII
1	6.53	15.29	31.56	52.76	71.34	83.30	88.45	89.01
2	5.79	10.35	18.93	31.29	48.95	79.88	86.43	90.00
3	5.80	11.08	23.32	47.96	61.52	75.25	83.59	91.89
4	6.53	9.68	17.47	29.19	52.85	79.88	86.70	90.11
5	7.43	12.91	33.05	54.25	72.50	84.81	91.08	91.39
6	7.15	13.43	29.75	55.02	70.63	80.23	89.21	92.07
7	10.35	15.31	32.00	64.45	75.08	84.78	92.17	92.53
8	9.67	13.22	30.11	63.01	73.32	82.54	91.51	92.14

Table VI. Percent Mortality (24 h) of Houseflies at Weekly Intervals When Fed with Controlled-Release Complexes

compd	weeks																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
diazinon (conv)	100	100	100	100	90	70	60	45	40	25	25	20	20	20	20	10	10	10	10	10	10	10	10	10	10
1	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	90	75	60	50	50
2	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	90	80	70	60	55
chlorpyrifos (conv)	100	100	100	100	100	100	100	100	90	90	80	75	70	60	60	55	50	50	50	40	40	30	30	30	30
3	95	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	90	75	60
4	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	95	90	80

at 420–435 cm^{-1} in complexes 5–8. The ν(M–S), ν(M–N), and ν(M–O) bands confirm the bidentate chelation of pesticides with the metal through S and N (1–4), S and O (5, 6), and N and O (7, 8).

The XRD patterns of pesticide–metal(II)–montmorillonite complexes 1–8 exhibit d_{001} spacing in the region 16.365–17.327 Å, and these peaks were attributed to complexation to the ligand (Table III). The increase in interlamellar spacing (8–9 Å) clearly indicates the complexation of pesticides with metal (1:1) in the interlamellars of the montmorillonite.

The RT ESR powder spectra of complexes 2 and 4 were characteristic of two g values, and those of 6 and 8 were characteristic of three g values (Table IV). Hence, the crystal field of copper in these complexes was distorted-tetragonal (axial symmetry) and orthorhombic, respectively (Kneubuhl, 1960).

Thus, the analytical and spectral data of these complexes confirm that Cu(II) in octahedral coordination with water occupying two of vacant sites was present in the interlamellars of montmorillonite. Similar coordination was expected for Co(II) complexes, present in the interlamellars of montmorillonite.

Chemical Assay. The pesticide–metal(II)–montmorillonite complexes were placed in water to study the release rate pattern (rate of cleavage of the pesticide–metal bond) under static laboratory conditions (Coppedge et al., 1975), i.e. the release of free pesticide into water. Table V shows the release rate patterns of clay complexes 1–8. Release was followed for an 8-week period for all the clay complexes. No metal leaching was observed from the clay complexes. The availability of the pesticide at the end of each week differs from complex to complex. All the pesticide–clay complexes released reasonable amounts of pesticide up to week 7, and more significant release of pesticide was between weeks 3–6. The amount of pesticide released at the end of week 8 was very small and may not be significant with the exception of complex 3. The release rate experiments were terminated at the end of week 8, since the release of pesticide was very insignificant. On the other hand, the adsorbed pesticides were totally leached from montmorillonite within 24 h. All the intercalated pesticides were stable for more than 2 years on the shelf. These results were very impressive in the sense that chemically pure diazinon tends to decompose on storage.

Bioassay. The insecticides diazinon and chlorpyrifos of conventional formulations and controlled-release complexes 1–4 were tested against the housefly for general and persistent toxicity. The activity of diazinon complexes 1 and 2 presented 100% toxicity upto week 20, while conventional diazinon showed 100% toxicity only up to week 4. Complexes 3 and 4 also showed the same 100% mortality for a prolonged time of 22 weeks, but the mortality started decreasing in conventional chlorpyrifos by week 8 (Table VI).

Thus, the chemical and bioassay results clearly demonstrated the increase of persistence of these pesticides upon encapsulation in the interlamellars of the montmorillonite. On the basis of these results, the prolonged bioactivity in agriculture can be safely predicted.

Applications of these encapsulated complexes as such as dusts and dust concentrates or as formulated granules and wettable powders for agricultural purposes have advantage of higher persistence over conventional formulations. Further, this is a convenient method especially for converting liquid pesticides having ligated atoms into solids. The present technique, which envisages the use of montmorillonite a cheap, abundantly available and an uncontaminating material for encapsulation of pesticides via complexation, can become potential alternative to the well-established immobilized methods.

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Sesquiterpenes in Glandular Trichomes of a Wild Tomato Species and Toxicity to the Colorado Potato Beetle

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Zingiberene, a sesquiterpene associated with resistance to the Colorado potato beetle (*Leptinotarsa decemlineata* Say) in *Lycopersicon hirsutum* f. *hirsutum* Humb. and Bonpl., occurred in the glandular tips of the type VI trichomes of this tomato species and was not present in the type IV or other trichomes or in the leaf matrix. Thus, transfer of the capacity to accumulate zingiberene into the cultivated tomato (which already has type VIs but lacks type IVs) will not be complicated by a requirement for multiple genes conferring high type IV densities. An alternative source of sesquiterpenes, the essential oil from roots of ginger (*Zingiber officinale* Roscoe), provided an extract containing predominantly zingiberene and smaller amounts of two sesquiterpenes tentatively identified as curcumene and bisabolene, in quantities sufficient for bioassays. This extract was toxic to CPB larvae at an LD_{50} at 7 μg of sesquiterpene/larva, a level provided by type VI trichomes occupying only 10–20 mm^2 of leaflet surface.

Lycopersicon hirsutum f. *hirsutum* Humb. and Bonpl. (*hir*) is resistant to a number of arthropod pests (Carter and Snyder, 1985; Juvik et al., 1982; Rick, 1982) but is reportedly susceptible to Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Fery and Kennedy, 1987). However, an *hir* accession with which we have worked exhibits sporadic resistance to CPB. Our *hir* does not contain the methyl ketone 2-tridecanone, which confers resistance to CPB and other insects in the related subspecies *L. hirsutum* f. *glabratum* C.H. Mull (*gla*) (Kennedy and Sorenson, 1985), but we have identified the sesquiterpene zingiberene in foliage extracts of *hir* by GC–MS (Carter et al., 1989). Zingiberene is occasionally found at high levels in *hir* leaves (Carter et al., 1989; Snyder et al., 1987) and appears to confer CPB resistance to *hir* and to *gla* × *hir* progeny segregating for zingiberene and tridecanone contents (Carter et al., 1989). Zingiberene content is variable in *hir* (Carter et al., 1989; Snyder and Hyatt, 1984b). However, zingiberene and related sesquiterpenes occur as major components of ginger root essential oil (Chen and Ho, 1988; Nigam et al., 1984), which would be a convenient source of sesquiterpenes for bioassay on CPB.

The location of terpenes implicated in insect resistance has often been ascribed to glandular trichomes located on the leaf surfaces of many plants (Duffey, 1986; Levin, 1973; Loomis and Croteau, 1980). Several types of trichomes are present on leaves of *Lycopersicon*, and species differ with respect to the types and densities of trichomes. For example, the type VI trichome, which has a four-celled glandular tip, is present at comparable densities on leaves of *hir*, *gla*, and the cultivated tomato, *Lycopersicon esculentum* (Mill.) (*esc*); but the type IV trichome, having a single glandular tip cell, is present on leaves of *hir* and *gla* but not on *esc* (Luckwill, 1943; Snyder and Carter, 1984a). Unidentified sesquiterpenes were found in the type VI trichomes of *L. hirsutum* (Lin et al., 1987), but at much lower concentrations per leaf than we have found (Carter et al., 1989). Snyder and Hyatt (1984) assigned zingiberene to type VI trichomes but did not distinguish between type VI and other trichomes in the collection of their extracts. Knowledge of the specific trichome in which zingiberene resides is important in transferring high zingiberene contents to the cultivated tomato, because if zingiberene occurs in the type VI trichome, rather than or in addition to the ubiquitous type VI, the transfer of zingiberene from *hir* to *esc* would be complicated by the necessity to transfer multiple genes conferring high type IV densities (Carter and Snyder, 1986). Thus, it is important to determine the specific trichome(s) in which zingiberene accumulates.

Our objectives were to determine whether zingiberene occurs specifically in *hir* trichomes and, if so, in which class of trichomes, and to assay the effect of zingiberene and

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