

# Enhanced Degradation and the Comparative Fate of Carbamate Insecticides in Soil

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Laboratory experiments investigated the comparative degradation of five carbamate insecticides in soil as affected by enhanced microbial degradation. Soils with prior field exposure to carbofuran, cloethocarb, or several carbamates contained adapted microbial populations capable of rapidly degrading carbofuran. Bendiocarb was rapidly degraded in all soils displaying enhanced carbofuran degradation, but carbaryl and cloethocarb were most rapidly degraded only in soil with prior exposure to several carbamates or to cloethocarb. The persistence of aldicarb and its oxidative metabolites aldicarb sulfoxide and aldicarb sulfone was not dramatically altered in soils with enhanced carbofuran degradation. Results indicate that although cross-adaptations for enhanced degradation exist within the carbamate insecticide class, structural similarity may play a role in modifying the expression of enhanced degradation in soil.

Enhanced degradation is the phenomenon whereby a soil-applied pesticide is rapidly degraded by a population of microorganisms that has adapted because of previous exposure to it or a similar pesticide. The result of this enhanced degradation may be a failure of the pesticide to adequately control the target pest due to dramatically decreased persistence (Felsot et al., 1982; Harvey et al., 1986). Pesticide catabolism by adapted soil microorganisms is microbially beneficial in that the pesticide or a hydrolysis product serves as a microbial carbon, energy, or nutrient source (Fournier et al., 1981; Karns et al., 1986; Racke and Coats, 1987; Tam et al., 1987). Enhanced degradation has been reported for a number of insecticides (Sethunathan and Pathak, 1972; Felsot et al., 1981; Read, 1983, 1987; Racke and Coats, 1987), herbicides (Newman and Thomas, 1949; Audus, 1951; Kirkland and Fryer, 1972; Wilson, 1984; Gray and Joo, 1985; Skipper et al., 1986), and fungicides (Walker et al., 1986; Yarden et al., 1986).

Carbofuran is a carbamate insecticide that has been widely applied to soil to control insect pests of corn, most notably larval corn rootworms (*Diabrotica* spp). Decreased persistence of carbofuran in fields with prior carbofuran exposure (Greenhalgh and Belanger, 1981) led to the discovery that enhanced microbial degradation was responsible for the decreased persistence and associated control failures observed (Felsot et al., 1981, 1982, 1985). Application of carbofuran to soil results in the appearance of a bacterial population capable of rapidly metabolizing subsequent applications of carbofuran (Felsot et al., 1981; Karns et al., 1986; Chaudhry et al., 1986). In addition to carbofuran, two other carbamate insecticides, carbaryl (Rodriguez and Dorrough, 1977) and aldicarb (Read, 1987), are also susceptible to enhanced degradation in soil.

There has been much interest in determining the specificity of the microbial adaptation involved in enhanced degradation. In some cases, microbial adaptation to rapidly degrade a specific pesticide in soil has resulted in the ability to degrade a variety of related pesticides (Audus, 1951; McClure, 1972; Kaufman and Edwards, 1983; Skipper et al., 1986), whereas in other cases the adaptation has been more narrowly confined to a single pesticide (Sethunathan and Pathak, 1972; Forrest et al., 1981; Racke and Coats, 1988; Read, 1988). Repeated laboratory treatments of soil with carbofuran were reported to induce rapid

degradation of carbofuran and a number of other carbamate insecticides (Harris et al., 1984). A carbofuran-degrading bacterium isolated from a soil column percolated with 200 ppm carbofuran has been shown to be capable of metabolizing several other carbamate insecticides (Karns et al., 1986).

The purpose of the present study was to investigate the specificity of enhanced carbamate degradation in soil as induced by normal field applications. A comparative approach examined the metabolism of a series of carbamate insecticides in the laboratory in soils with confirmed enhanced carbofuran degradation and in companion soils taken from adjacent fields with no recent insecticide history. Insecticides chosen for study were carbofuran, bendiocarb, carbaryl, cloethocarb, and aldicarb (Figure 1).

## MATERIALS AND METHODS

**Chemicals.** The following radiolabeled insecticides, along with model metabolites, were obtained from the respective sources: [*ring*-<sup>14</sup>C]carbofuran and [*carbonyl*-<sup>14</sup>C]carbofuran, FMC Corp., Princeton, NJ; [2-*benzodioxole*-<sup>14</sup>C]bendiocarb, Fisons Corp., Bedford, MA; [1-*naphthyl*-<sup>14</sup>C]carbaryl, Nuclear Chicago, Chicago, IL; [*phenyl*-4-<sup>14</sup>C]cloethocarb, BASF Corp., Parsippany, NJ; [*methylthio*-<sup>14</sup>C]aldicarb, California Bionuclear Corp., Sun Valley, CA. All compounds were of greater than 98% radiopurity. Chemical structures of these insecticides are shown in Figure 1.

**Soils.** Surface samples of soils (Table I) were collected from cornfields at the end of the growing season, sieved to remove debris, and stored in a moist condition at 4 °C. Two soils (II, III) had received a carbofuran application the previous two growing seasons. A companion soil (I) came from an adjacent field with no recent insecticide history. Two soils were from fields on an experimental farm: one soil (IV) with no recent insecticide history and the other soil (V) with extensive insecticide history. The soil with extensive insecticide history had carbofuran applied the previous year and mixed carbamate and organophosphorus insecticide treatments every other year for 10 years with alternate years untreated. Carbamate insecticides previously used on soil V included carbofuran, bendiocarb, and cloethocarb. The final pair of soils came from within one field: one soil (VI) from a plot with no recent insecticide history and the other soil (VII) from a plot that had received an annual treatment of cloethocarb for each of the last 4 years. The last field application of carbofuran and cloethocarb failed to provide adequate control of corn rootworms (*Diabrotica* spp.) in soils II, III, V, and VII, respectively. Characteristics of soils used in

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Table I. Soils Used in the Comparative Insecticide Degradation Study

no.	soil series <sup>a</sup>	insecticide history <sup>b</sup>	pH	OM	texture			% carbofuran <sup>c</sup> degraded in 1-week assay
					sand	silt	clay	
I	Bourbon	none	6.4	8.1	56.6	26.0	17.4	17.8
II	Bourbon	carbofuran	6.7	3.7	63.8	20.5	15.7	91.1
III	Tama	carbofuran	7.0	4.4	8.6	58.7	32.7	85.2
IV	Readlyn	none	7.0	3.9	41.3	34.7	24.0	19.7
V	Readlyn	carbamates	6.7	4.3	29.3	41.3	29.4	81.5
VI	Webster	none	6.6	4.9	34.6	36.0	29.4	24.5
VII	Webster	cloethocarb	7.2	5.1	37.8	34.8	27.4	86.6

<sup>a</sup>Soils I and II (Bourbon), soils IV and V (Readlyn), and soils VI and VII (Webster) were companion soils taken from adjacent fields. <sup>b</sup>For soils with no insecticide history (I, IV, VI), includes last 10 years; soils II and III, carbofuran applied previous 2 years; soil VII, cloethocarb applied previous 4 years; soil V, received carbofuran previous year and mixed carbamate and organophosphorus insecticides every other year for 10 years, with alternate years untreated. <sup>c</sup>A 25-g aliquot of each soil, in duplicate, was treated with 5 ppm [*carbonyl-<sup>14</sup>C]carbofuran (0.1  $\mu$ Ci), mixed with 75 g of sand, and incubated for 1 week. Percent carbofuran degraded is based on the quantity of <sup>14</sup>CO<sub>2</sub> evolved. Results are means of duplicate tests.*

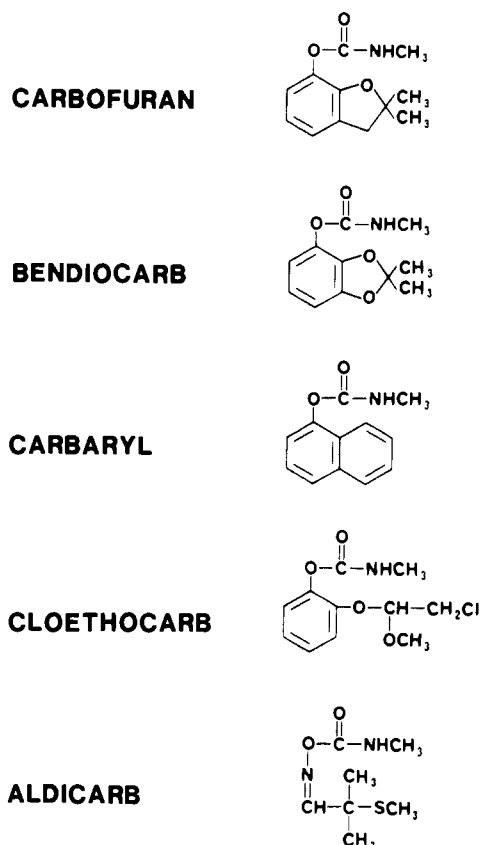


Figure 1. Structures of carbamate insecticides used in the comparative degradation study.

the comparative degradation study are listed in Table I.

Confirmation that the adaptation resulting in enhanced carbofuran degradation had occurred in the carbamate history soils (II, III, V, VII) was made by using an assay of [*carbonyl-<sup>14</sup>C]carbofuran degradation to <sup>14</sup>CO<sub>2</sub>, demonstrating microbial involvement, and enumerating adapted, carbofuran-degrading microorganisms according to the methodology of Racke and Coats (1987).*

**Soil Insecticide Treatment.** The seven soils used in the study (Table I) were treated separately with each of the five [<sup>14</sup>C]insecticides at 5 ppm (1.0  $\mu$ Ci) in acetone (Lichtenstein and Schulz, 1959) in duplicate 100-g samples and added to 250-mL glass jars (5-cm diameter  $\times$  11-cm height). [*ring-U-<sup>14</sup>C]Carbofuran was used for this study. Soils were moistened to field capacity (<sup>1</sup>/<sub>3</sub> bar soil moisture tension) with distilled water and incubated at 25 °C in the dark by using a flow-through incubation system (Ferris and Lichtenstein, 1980). Air was periodically purged from the tightly sealed jars through both vapor and CO<sub>2</sub> traps, which*

allowed maintenance of aerobic conditions and monitoring of [<sup>14</sup>C]insecticide degradation to <sup>14</sup>CO<sub>2</sub>.

**Extraction and Analyses.** After a 4-week incubation, [<sup>14</sup>C]insecticide residues in soil were extracted twice with acetone-methanol (1:1) and once with acetone-methanol-dichloromethane (1:1:1) and partitioned into dichloromethane as described by Lichtenstein et al. (1973). Thin-layer chromatographic (TLC) and autoradiographic systems were used to characterize carbofuran (Metcalf et al., 1968), bendiocarb (Hsin and Coats, 1988), carbaryl (Leeling and Casida, 1966), aldicarb (Bull et al., 1967), and their respective metabolites. Organic-soluble extracts of cloethocarb-treated soils were spotted on silica gel TLC plates and developed in chloroform-ethyl acetate-*n*-hexane (5:2:1) (cloethocarb *R<sub>f</sub>* 0.53). Qualitative confirmation of the identity of parent insecticides was by gas-liquid chromatography, using a Varian 3740 chromatograph equipped with a 10% DC-200/2% OV-225 on 80/100-mesh Chromosorb column (2 mm  $\times$  90 cm) and a TSD (N-P) detector. Unextractable, soil-bound [<sup>14</sup>C]insecticide residues were recovered by combustion to <sup>14</sup>CO<sub>2</sub> in a Packard sample oxidizer.

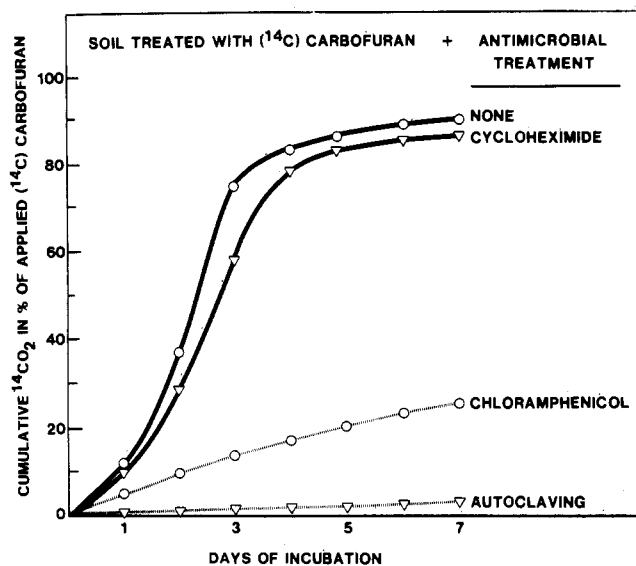
## RESULTS

**Confirmation of Carbofuran-Enhanced Degradation.** The [*carbonyl-<sup>14</sup>C]carbofuran degradation assay (Table I) revealed that carbofuran was rapidly degraded in soils with prior carbofuran exposure (II, III, V). This rapid degradation was also evident in the soil with cloethocarb history (VII). Results clearly divided the soils into two categories: those with prior carbamate exposure that degraded 82–91% of the applied carbofuran (II, III, V, VII) in 1 week and those with no recent insecticide exposure that degraded 18–25% of the applied carbofuran (I, IV, VI). The evolution rate of <sup>14</sup>CO<sub>2</sub> from the carbamate history soils accelerated during the first few days of incubation (e.g., soil II, Figure 2), which is indicative of enhanced microbial degradation (Kaufman and Edwards, 1983; Racke and Coats, 1987). Sterilization or application of bactericide (chloramphenicol) inhibited the degradation of carbofuran, implicating microbial involvement. A most-probable-number assay conducted with fresh soil samples indicated that between  $1.5 \times 10^5$  and  $7.4 \times 10^5$  carbofuran-degrading microorganisms were present per gram of carbamate history soil (II, III, V, VII). In contrast, an average of  $0.5 \times 10^5$  carbofuran degraders was detected in insecticide-free soils (I, IV, VI). There may also have been a qualitative difference between the carbofuran-degrading microorganisms in carbamate history and companion soils (e.g., rate of carbofuran metabolism) because the quantitative difference alone was not of the magnitude one would associate with such a dramatic difference in soil carbofuran degradation rate. However, all lines of evidence*

**Table II. Effect of Insecticide History on the Fate and Degradation of [<sup>14</sup>C]Insecticides in Soil during a 4-Week Incubation<sup>a</sup> (Results Expressed as <sup>14</sup>C Recovered in Percent of Applied [<sup>14</sup>C]Insecticide)**

fraction	soil insecticide history <sup>b</sup> (no.)						
	none I	carbofuran II	carbofuran III	none IV	carbamates V	none VI	cloethocarb VII
	Carbofuran						
carbofuran	29.4 <sup>d</sup>	0.9 <sup>e</sup>	1.2 <sup>e</sup>	56.0 <sup>f</sup>	0.8 <sup>e</sup>	56.6 <sup>f</sup>	1.6 <sup>e</sup>
3-ketocarbofuran	3.4	0.3	0.3	4.5	0.2	1.4	0.4
other <sup>c</sup>	3.1	1.6	1.1	2.0	1.7	1.2	1.8
soil bound	41.9 <sup>d</sup>	69.2 <sup>e</sup>	60.3 <sup>f</sup>	29.5 <sup>g</sup>	71.8 <sup>eh</sup>	27.2 <sup>g</sup>	76.9 <sup>h</sup>
<sup>14</sup> CO <sub>2</sub>	13.8 <sup>d</sup>	30.4 <sup>e</sup>	36.4 <sup>f</sup>	5.8 <sup>g</sup>	14.0 <sup>d</sup>	8.6 <sup>h</sup>	11.0 <sup>i</sup>
total	91.5 <sup>de</sup>	102.3 <sup>d</sup>	99.3 <sup>de</sup>	97.8 <sup>de</sup>	88.5 <sup>e</sup>	94.9 <sup>de</sup>	91.7 <sup>de</sup>
	Bendiocarb						
bendiocarb	34.6 <sup>d</sup>	0.9 <sup>e</sup>	1.6 <sup>e</sup>	30.4 <sup>f</sup>	1.3 <sup>e</sup>	47.2 <sup>g</sup>	0.9 <sup>e</sup>
other <sup>c</sup>	3.5	2.7	2.1	2.0	1.9	2.3	2.1
soil bound	38.5 <sup>d</sup>	43.4 <sup>e</sup>	51.9 <sup>f</sup>	38.4 <sup>d</sup>	42.4 <sup>e</sup>	28.3 <sup>g</sup>	51.8 <sup>f</sup>
<sup>14</sup> CO <sub>2</sub>	19.5 <sup>d</sup>	42.4 <sup>e</sup>	36.2 <sup>f</sup>	23.3 <sup>g</sup>	43.2 <sup>e</sup>	18.7 <sup>d</sup>	36.2 <sup>f</sup>
total	96.0	89.4	91.9	94.1	88.8	96.5	91.1
	Carbaryl						
carbaryl	26.8 <sup>d</sup>	17.7 <sup>e</sup>	42.6 <sup>f</sup>	38.6 <sup>g</sup>	5.8 <sup>h</sup>	54.1 <sup>i</sup>	9.3 <sup>h</sup>
other <sup>c</sup>	0.2	0.1	2.7	0.1	0.0	0.2	0.1
soil bound	8.6 <sup>d</sup>	6.8 <sup>e</sup>	11.3 <sup>f</sup>	7.9 <sup>de</sup>	4.8 <sup>g</sup>	10.6 <sup>f</sup>	7.4 <sup>de</sup>
<sup>14</sup> CO <sub>2</sub>	55.3 <sup>d</sup>	68.2 <sup>e</sup>	42.2 <sup>f</sup>	46.9 <sup>g</sup>	80.6 <sup>h</sup>	22.9 <sup>i</sup>	80.0 <sup>h</sup>
total	90.9 <sup>df</sup>	92.8 <sup>de</sup>	97.2 <sup>e</sup>	93.5 <sup>de</sup>	91.2 <sup>df</sup>	87.8 <sup>f</sup>	96.8 <sup>e</sup>
	Cloethocarb						
cloethocarb	48.3 <sup>d</sup>	24.0 <sup>e</sup>	41.1 <sup>f</sup>	50.0 <sup>d</sup>	1.2 <sup>g</sup>	67.0 <sup>h</sup>	2.6 <sup>g</sup>
other <sup>c</sup>	1.3 <sup>d</sup>	2.1 <sup>e</sup>	1.1 <sup>d</sup>	1.2 <sup>d</sup>	1.0 <sup>d</sup>	2.0 <sup>e</sup>	0.9 <sup>d</sup>
soil bound	31.3 <sup>d</sup>	43.9 <sup>e</sup>	41.3 <sup>e</sup>	32.1 <sup>d</sup>	67.0 <sup>f</sup>	22.8 <sup>g</sup>	67.7 <sup>f</sup>
<sup>14</sup> CO <sub>2</sub>	14.1 <sup>d</sup>	15.3 <sup>d</sup>	12.4 <sup>e</sup>	9.3 <sup>f</sup>	21.7 <sup>g</sup>	8.1 <sup>f</sup>	18.2 <sup>h</sup>
total	95.0 <sup>de</sup>	85.2 <sup>e</sup>	95.9 <sup>de</sup>	92.8 <sup>de</sup>	90.9 <sup>de</sup>	99.9 <sup>d</sup>	89.3 <sup>de</sup>
	Aldicarb						
aldicarb	1.1	1.6	0.6	0.6	0.5	0.5	1.4
aldicarb sulfoxide	3.1 <sup>d</sup>	16.4 <sup>e</sup>	11.5 <sup>fg</sup>	10.7 <sup>g</sup>	11.9 <sup>fg</sup>	13.6 <sup>f</sup>	12.0 <sup>fg</sup>
aldicarb sulfone	17.6 <sup>d</sup>	17.8 <sup>d</sup>	18.1 <sup>d</sup>	18.4 <sup>d</sup>	7.3 <sup>e</sup>	20.3 <sup>f</sup>	12.9 <sup>g</sup>
other <sup>c</sup>	7.5 <sup>d</sup>	16.0 <sup>e</sup>	11.7 <sup>f</sup>	13.7 <sup>ef</sup>	12.0 <sup>f</sup>	11.1 <sup>f</sup>	13.3 <sup>ef</sup>
soil bound	19.8 <sup>d</sup>	15.3 <sup>e</sup>	19.9 <sup>d</sup>	20.6 <sup>d</sup>	24.3 <sup>f</sup>	19.9 <sup>d</sup>	21.7 <sup>d</sup>
<sup>14</sup> CO <sub>2</sub>	38.2 <sup>d</sup>	29.6 <sup>e</sup>	33.7 <sup>df</sup>	31.8 <sup>ef</sup>	37.7 <sup>d</sup>	30.7 <sup>e</sup>	35.2 <sup>df</sup>
total	87.3 <sup>d</sup>	96.7 <sup>e</sup>	95.5 <sup>e</sup>	95.8 <sup>e</sup>	93.7 <sup>e</sup>	96.1 <sup>e</sup>	96.6 <sup>e</sup>

<sup>a</sup> [<sup>14</sup>C]Insecticides were each uniformly applied to duplicate 100-g portions of soil at 5 ppm (1.0 μCi). <sup>b</sup> Soil insecticide history: previous 10 years for I, IV, and VI; previous 2 years for soils II and III; previous 4 years for soil VII; soil V received carbofuran previous year and mixed carbamate and organophosphorus insecticides every other year for 10 years, with alternate years untreated. <sup>c</sup> Primarily water-soluble metabolites with traces of organic-soluble metabolites. <sup>d-i</sup> Means followed by the same letter in each horizontal row are not significantly different at the 1% level (Student-Newman-Keuls test).



**Figure 2.** Degradation of [*carbonyl*-<sup>14</sup>C]carbofuran to <sup>14</sup>CO<sub>2</sub> in a carbofuran history soil (II) as affected by antimicrobial treatments. Results are means of duplicate tests.

confirmed the occurrence of enhanced carbofuran degradation in the carbamate history soils.

**Comparative Degradation of Carbamate Insecticides in Soil.** The comparative degradation of the five

carbamate insecticides was evaluated in each of the seven soils to determine which of the other carbamate insecticides would also be rapidly degraded in soil displaying enhanced carbofuran degradation. Results of the comparative degradation study are presented in Table II and will be summarized for each insecticide.

As anticipated by the [*carbonyl*-<sup>14</sup>C]carbofuran degradation assay, very little [*ring*-U-<sup>14</sup>C]carbofuran persisted after the 4-week incubation in soils with prior carbamate exposure (II, III, V, VII). In contrast, between 29 and 57% of the initially applied carbofuran remained in the companion soils (I, IV, VI). The rapid degradation of carbofuran resulted in the production of large quantities of soil-bound residues.

*Bendiocarb* persistence in soil was similarly affected by prior carbamate exposure. In all soils in which carbofuran had been rapidly degraded, *bendiocarb* was likewise rapidly degraded and only about 1% of the initially applied *bendiocarb* persisted in these soils (II, III, V, VII) after 4 weeks. In contrast, between 30 and 47% of the applied *bendiocarb* persisted in the companion soils (I, II, VI). Rapid degradation of *bendiocarb* resulted in increased production of soil-bound residues and also in increased production of <sup>14</sup>CO<sub>2</sub> in comparison with companion soils.

*Carbaryl* degradation results were mixed in the carbamate history soils. In one carbofuran history soil (II), *carbaryl* was slightly less persistent and, in another soil (III), markedly more persistent than in the companion soil

(I). However, carbaryl degradation was much accelerated in soil with mixed-carbamate history (V) and in soil with cloethocarb history (VII). The increased degradation of carbaryl in these two soils (V, VII) resulted in the production of large quantities of  $^{14}\text{CO}_2$ , which by the second week of incubation accounted for 72 and 65% of the applied [ $^{14}\text{C}$ ]carbaryl, respectively.

The degradation of *cloethocarb* was also only moderately altered in soils with carbofuran history. Although significantly less cloethocarb persisted in both carbofuran history soils (II, III) than in the companion soil (I), the difference observed was much less than that observed with carbofuran or bendiocarb. As with carbofuran, bendiocarb, and carbaryl, cloethocarb was rapidly degraded in the carbamate history soil (V). Rapid degradation of cloethocarb was also observed in soil with prior exposure to cloethocarb (VII). The rapid degradation of cloethocarb in soils V and VII resulted in greater formation of soil-bound residues and slightly greater  $^{14}\text{CO}_2$  production than that observed in companion soils (IV, VI).

*Aldicarb* was not very persistent in any of the soils tested, with less than 2% of applied aldicarb remaining after the 4-week incubation in each soil. Aldicarb was instead represented by its insecticidal metabolites aldicarb sulfoxide and aldicarb sulfone. The quantities of aldicarb sulfoxide recovered from the carbamate history soils were not significantly less than those recovered from the corresponding companion soils. This was not true for aldicarb sulfone, of which significantly less was present in the carbamate history soil (V) and the cloethocarb history soil (VII) than in the companion soils (IV, VI). These decreases in aldicarb sulfone present were accompanied by corresponding increases in  $^{14}\text{CO}_2$  and soil-bound residue production in relative comparison to the companion soils (IV, VI).

## DISCUSSION

Evidence from the current investigation clearly suggests that cross-adaptations for enhanced degradation occur within the carbamate insecticide class. Previous exposure of soil to carbofuran, cloethocarb, or several carbamates induced the rapid degradation of carbamate compounds that had not been previously applied to a given soil. Evidence for induction of relatively nonspecific enhanced carbamate degradation in soil has been reported from soils exposed to carbofuran in the field (Kaufman and Edwards, 1983; Felsot, 1986) and soils subject to repeated carbofuran applications in the laboratory (Harris et al., 1984; Chapman et al., 1986b; Felsot, 1986). However, most of the previous laboratory studies in which enhanced degradation was induced by repeated laboratory treatments did not distinguish between cometabolism and true cross-adaptation of the subsequently applied carbamates. Cross-adaptation would imply the ability of a compound to induce the appropriate microbial response (e.g., enzyme production) in the absence of large quantities of previous substrate. Cross-adaptations for enhanced degradation have also been reported within the phenoxyacetic acid herbicide class (Newman and Thomas, 1949; Audus, 1951; Kirkland and Fryer, 1972) and within the thiocarbamate herbicide class (Wilson, 1984; Skipper et al., 1986; Harvey et al., 1986). This is in contrast to the lack of cross-adaptations associated with enhanced degradation within the organophosphorus insecticide class as expressed in soil (Sethunathan and Pathak, 1972; Forrest et al., 1981; Racke and Coats, 1988; Read, 1988). In addition, enhanced carbofuran degradation does not affect the degradation of organophosphorus insecticides (Racke and Coats, 1988), thiocarbamate herbicides (Kaufman and Edwards, 1983),

or dithiocarbamate herbicides (Harris et al., 1984) in soil.

Also evident from the current investigation is that not all the carbamates were as rapidly degraded as carbofuran in soil displaying enhanced carbofuran degradation. It is possible that similarity in chemical structure may play a role in moderating the cross-adaptations expressed. Bendiocarb, a carbamate that is extremely similar in structure to carbofuran (Figure 1), was rapidly degraded in each soil that rapidly degraded carbofuran. With carbaryl and cloethocarb, this was not the case, however, because in soils exposed previously only to carbofuran, the increase in carbaryl or cloethocarb degradation was slight or absent. Felsot (1986) reported that although bendiocarb, trimethacarb, and isoprocarb were all rapidly degraded in soil with enhanced carbofuran degradation, bufencarb was not. Similarly, Read (1987) found that soil repeatedly treated with carbofuran in the laboratory developed the capacity to degrade carbofuran, propoxur, and cloethocarb rapidly, but not oxamyl or aldicarb. Chapman et al. (1986b) determined that although one laboratory pretreatment of soil with carbofuran induced rapid degradation of both carbofuran and carbaryl, four pretreatments were needed to induce rapid cloethocarb degradation and oxamyl was not rapidly degraded even after nine carbofuran pretreatments. A range of selectivity of cross-adaptations involved in enhanced degradation has also been reported within the phenoxyacetic acid herbicide class (Kirkland and Fryer, 1972) and the thiocarbamate herbicide class (Wilson, 1984; Skipper et al., 1986; Harvey et al., 1986).

The idea that a range of intensities of cross-adaptations exists within the carbamate insecticide class is supported by work with a carbofuran-degrading bacterium isolated from soil. Karns et al. (1986) reported that an *Achromobacter* isolated from carbofuran-treated soil could hydrolyze not only carbofuran but also several other carbamates. However, the rate of hydrolysis depended on similarity of structure to carbofuran, with hydrolysis rates for the following compounds arranged in decreasing order: carbofuran > carbaryl > propoxur > aldicarb > *O*-nitrophenyl dimethylcarbamate. Work conducted with carbofuran hydrolase, the carbofuran-hydrolyzing enzyme isolated from this *Achromobacter*, revealed that although this enzyme was very efficient in hydrolyzing carbofuran and carbaryl, it was much less efficient in hydrolyzing aldicarb *in vitro* (Derbyshire et al., 1988).

It is possible that both inherent and induced soil properties may play a role in modulating the extent to which carbamate cross-adaptations are expressed. Factors such as soil pH, moisture, organic-matter content, and temperature have been shown to affect the development and expression of enhanced carbofuran degradation (Read, 1983; Chapman et al., 1986a,b). These same factors may also affect the magnitude of the cross-adaptations observed. Chapman et al. (1986b) reported that soils collected from the same plots but during different seasons displayed different degrees of carbamate cross-adaptations in response to repeated laboratory carbofuran treatments. Although the ecology of carbofuran-degrading microorganisms in soil has not been examined, members of three bacterial genera have been implicated in enhanced carbofuran degradation (Felsot et al., 1981; Karns et al., 1986; Chaudhry et al., 1986). It is not known whether specific bacterial species or species complexes display similar selectivity or even genetic bases for carbamate metabolism. In the current investigation, carbofuran, bendiocarb, carbaryl, and cloethocarb were rapidly degraded in soil with prior exposure to various carbamates, yet only carbofuran

and bendiocarb were unequivocally subject to enhanced degradation in soils previously exposed only to carbofuran. It is possible that different populations of carbamate-degrading microorganisms may have been induced, depending on the range of substrates to which the soil had previously been exposed. Prior use of cloethocarb induced rapid degradation of carbofuran in the current investigation, but prior use of carbofuran only slightly accelerated cloethocarb degradation. Cloethocarb persistence in soil has previously been reported to be unaffected by enhanced carbofuran degradation (Hamm and Thomas, 1986).

The susceptibility of carbamate insecticides to enhanced degradation is a significant development that may limit the long-term usefulness of these compounds in soil. Recognition of enhanced carbofuran degradation as the cause of pest control failures may be partly responsible for the substantial decrease in its use against corn rootworms in Iowa (Becker and Stockdale, 1980; Wintersteen and Hartzler, 1987). Enhanced degradation may also have played a substantial role in recent decisions to suspend development of bendiocarb and cloethocarb for this same market. Additional research regarding the microbial ecology and genetics of enhanced degradation, as well as factors that render particular pesticides or groups of pesticides susceptible to enhanced degradation, is needed.

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**Registry No.** Carbofuran, 1563-66-2; bendiocarb, 22781-23-3; carbaryl, 63-25-2; cloethocarb, 51487-69-5; aldicarb, 116-06-3.

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## Development of an Enzyme Immunoassay for Endosulfan and Its Degradation Products

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An enzyme immunoassay (EIA) was developed for the insecticide endosulfan and its degradation products. The EIA was based on antibodies raised against the diol of endosulfan by immunizing rabbits with a keyhole limpet hemocyanin (KLH) endosulfandiold conjugate. With this method, endosulfan was detected in aqueous solutions at a level of 3 ppb without any sample extraction procedure. The measuring range was found to be between 3 and 400 ng/mL. The cross-reactivity of other similar chlorinated hydrocarbon pesticides was tested. Whereas the pesticides lindan, alodan, and aldrin showed only little cross-reactivities, endrin demonstrated a high cross-reactivity of 180%.

Environmental analytical studies of endosulfan (Figure 1) and its degradation product residues have so far relied on conventional analytical techniques, in particular silica gel column chromatography and a subsequent quantitative determination by infrared measurement (Weinmann, 1970) or gas chromatography with a time-consuming cleanup stage (Zweig and Archer, 1960). Therefore, a simple and sensitive method would be a highly desirable aim. Possibly, an immunological procedure, which makes use of a highly specific antibody, could be suitable for a rapid, efficient, and sensitive analytical method.

Recently, immunological methods such as enzyme-linked immunosorbent assay were shown to be applicable to the field of environmental analytical chemistry. The application of immunological methods especially in the analysis of pesticides has been treated in great detail by Hammock

and Mumma (1980) and reviewed by Schwalbe-Fehl (1986).

So far, the present study demonstrates the first development of an EIA method to detect a chlorinated hydrocarbon pesticide. The optimum experimental conditions for the determination and the sensitivity, precision, and specificity of the method are discussed.

### MATERIALS AND METHODS

**Chemicals.** Endosulfan and its degradation products (ether, lactone, diol, sulfate) were provided by Hoechst AG, Frankfurt. The pesticides alodan, aldrin, endrin, and lindan were supplied by Riedel-de Haen, Seelze. Bovine serum albumin, keyhole limpet hemocyanin, peroxidase, *o*-phenylenediamine dihydrochloride, Tween 20, Freund's adjuvant, dicyclohexylcarbodiimide, and carbonyldiimidazole were obtained from Sigma Chemie, Deisenhofen, and phosgen was purchased from Fluka.

**Buffer Solutions.** Coating buffer: pH 9.6, 50 mM Na<sub>2</sub>CO<sub>3</sub>. Phosphate-buffered saline with 0.1% Tween 20 (PBS-t): pH 7.4; prepared by adding NaCl (150 mM),

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