

Original Article

Bendiocarb Metabolism in Adults and Larvae of the Southern Corn Rootworm, *Diabrotica undecimpunctata howardi*

Chu-Ying HSIN and Joel R. COATS*

Taiwan Plant Protection Center, Taichung, Taiwan, Republic of China

*Department of Entomology, Iowa State University,
Ames, Iowa 50011, U.S.A.

(Received October 16, 1986)

The toxicity and metabolism of bendiocarb were compared between adults and larvae of southern corn rootworm (SCR), *Diabrotica undecimpunctata howardi* BARBER. The 48-hr topical LD₅₀ values of bendiocarb to SCR adults and larvae were 1.3 and 9.1 µg/g, respectively. Bendiocarb was approximately seven times less toxic to SCR larvae than to adults. In the metabolism studies, SCR adults and larvae were analyzed at specific time intervals after treatment with ¹⁴C-bendiocarb at sublethal 1/10 LD₅₀ dosage level. The major metabolites from bendiocarb were bendiocarb phenol and conjugated compounds for SCR adults and larvae, respectively. The proposed major metabolic pathways of bendiocarb in SCR were hydrolysis of the carbamate ester bond to yield bendiocarb phenol and the subsequent conjugation of bendiocarb phenol. The ratio of applied dose to internal toxic compound was similar for both adults and larvae. Therefore, the amount of internal toxic compound, affected by the rates of penetration, metabolism, and excretion, was not a determining factor in the selective toxicity of bendiocarb between SCR adults and larvae.

INTRODUCTION

Bendiocarb (Tatoo®, Ficom®, 2,2-dimethylbenzo-1,3-dioxol-4-yl *N*-methylcarbamate) is structurally similar to carbofuran (Fig. 1). It shows high contact and residual activity on a wide spectrum of insect species.¹⁾ Biological activity of bendiocarb has been studied in the Japanese beetle, *Popillia japonica* NEWMAN,²⁾ German cockroach, *Blattella germanica* (L.),³⁾ and *Anopheles stephensi* LISTON.⁴⁾ Toxicological reports include residual studies in corn⁵⁾ and metabolism studies in the rat and man.⁶⁾ The literature does not provide any description of bendiocarb metabolism in insects.

Corn rootworms, in the genus *Diabrotica*, feed on the roots of the corn plant as larvae and feed on the silks as adults (beetles). Insecticide treatments differ for the two stages, as does the susceptibility of each stage to

various insecticidal chemicals. Comparative toxicity of the larval and adult stages is also important because screening tests for susceptibility or resistance have traditionally been conducted with adults while it is the larvae that inflict greater economic losses. Bendiocarb is 7-fold more toxic to adult southern corn rootworm than to larvae by topical dosing.

Differential toxicity could be due to differences in site sensitivity, penetration, excretion, or metabolism in the two stages. This paper describes the toxicity and the fate of bendiocarb in southern corn rootworm (SCR), *Diabrotica undecimpunctata howardi* BARBER by comparing the distribution, penetration, and metabolic pathways of ¹⁴C-bendiocarb between SCR adults and larvae.

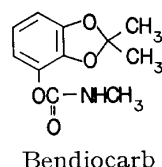


Fig. 1 Bendiocarb carbamate insecticide.

MATERIALS AND METHODS

1. Chemicals

^{14}C -Bendiocarb (labeled at the No. 2 carbon of benzodioxole ring), analytical-grade bendiocarb, and the metabolite, bendiocarb phenol (2,2-dimethylbenzo-1,3-dioxol-4-ol) were provided by Fisons Corp., Bedford, MA, U.S.A. The radiochemical purity of ^{14}C -bendiocarb was greater than 99% and had a specific activity of 9.95 mCi/mmol. Radiochemicals were dissolved in benzene (1 mg/ml) and stored in the dark at -21°C . Analytical standards of bendiocarb and bendiocarb phenol were dissolved in acetone to make 1% stock solutions. The stock solutions were refrigerated at 4.5°C .

2. Thin-layer Chromatography, Autoradiography, and Liquid Scintillation Counting

Bendiocarb and its metabolites were separated by one-dimensional thin-layer chromatography (TLC) using 5×20 -cm silica gel 60 F₂₅₄ plates (0.25-mm thickness) purchased from EM Science, Gibbstown, NJ. *R_f* values of bendiocarb and its metabolites in two solvent systems, toluene-ethyl acetate (4:1) and hexane-isopropanol (4:1), are shown in Table 1. Analytical standards were visualized on chromatograms by using ultraviolet (UV) light (254 nm).

Radioactive samples were examined with

Table 1 *R_f* values for bendiocarb and its metabolites in TLC systems.

Compound	Solvent system	
	Toluene-ethyl acetate (4:1)	Hexane-isopropanol (4:1)
Bendiocarb phenol	0.39	0.47
Bendiocarb	0.24	0.37
Unknown	0.11	0.30

analytical standards by using cochromatography. Radiolabeled spots corresponding to known standards on TLC plates were scraped into scintillation vials containing 10 ml of PPO-toluene cocktail (5 g PPO per liter of toluene). The radioactivity was counted by liquid scintillation counting (LSC). These spots were also located and confirmed by using autoradiography. This was accomplished by exposing Kodak Blue Brand X-ray film to the TLC plates for 2 to 4 weeks, then developing and fixing the film. Radioactivity was measured by an LKB Wallac® model 1217 RackBeta liquid scintillation counter. Counts were corrected for background and counting efficiency. Counting efficiency was determined by quench correction, using the external standard channels ratio method.

3. Bioassay

Toxicity for SCR adults and larvae was determined by using 48-hr topical LD₅₀s by previously described methods.⁷⁾ SCR were obtained from a laboratory colony and reared according to methods described by Branson *et al.*⁸⁾ Early third-instar larvae (12 to 16 mg) and adults (14 to 16 days old, 22 to 26 mg) were used for bioassay. Each test was conducted with insecticide acetone solutions on at least 180 insects. The experiment was a randomized complete-block design, composed of three replicates of 10 insects at each of five dosages and a control (solvent only). Adults were anesthetized with carbon dioxide to facilitate handling with forceps; larvae were manipulated with a vacuum suction tube.

Individual beetles were treated topically on the ventral abdominal region with 1 μl of insecticide solution. Treatments were administered using a microapplicator fitted with a calibrated 250- μl syringe (Hamilton Co., Reno, NV) and a 27-gauge hypodermic needle. Groups of treated beetles were kept in petri dishes containing water and lettuce pieces. Larvae were treated similarly and kept in petri dishes with water and germinated corn. Mortalities were recorded 48 hr after treatment. The LD₅₀ value and its standard error (SE) were computed by probit analysis from dosage-mortality data.^{9,10)}

4. Metabolism and Distribution of Bendiocarb

Each SCR received 1 μ l of ^{14}C -radiolabeled bendiocarb in acetone topically applied on the ventral abdomen. Adults were treated at the topical LD_{10} value (0.80 $\mu\text{g/g}$) and the 1/10 LD_{50} value (0.13 $\mu\text{g/g}$), and larvae were treated at the 1/10 LD_{50} value (0.91 $\mu\text{g/g}$). Each metabolism experiment was conducted with 50 SCR, except for the studies at 0.083 hr and 0.5 hr posttreatment when 20 SCR were used.

After treatment, SCR were placed in a 1-oz French square bottle with cotton plug. Insects were held at room temperature without food or water for the predetermined time period. No mortality was observed for insects treated at the 1/10 LD_{50} dose. Both adults and larvae were sacrificed by deep-freezing the bottle at posttreatment intervals of 0.083, 0.5, 1, 2, and 4 hr.

Frozen SCR were analyzed by using procedures adopted from Hollingworth *et al.*¹¹⁾ Each SCR was rinsed three times with 25 ml of acetonitrile. This fraction was designated "external rinse." Rinsed SCR were then homogenized in 25 ml of acetonitrile, and extracts were filtered by Buchner funnel. The extraction step was repeated three times. The three acetonitrile filtrates were pooled and partitioned with 20 ml of hexane to remove lipids and pigments. The acetonitrile phase (from partitioning the extracts) was referred to as the "internal organic extract." The residue was rehomogenized with 10 ml of distilled water, and the homogenate filtered through a Buchner funnel. This procedure was repeated three times, followed by extraction with 10 ml of acetone-methanol (1:1). All distilled water and acetone-methanol filtrates were combined to form the "internal aqueous extract." The residual extraction material was air-dried and digested by chemical solubilization techniques¹²⁾ to provide an "unextractable residue" fraction. Residues in holding jars were digested and solubilized similarly to the unextractable residue fractions, using perchloric acid, hydrogen peroxide, and 2-ethoxyethanol¹²⁾ to form the "container rinse" fractions.

Total radioactivity in all rinses, extracts, and residual fractions was assayed by LSC. The individual "external rinse" and "internal organic extract" fractions were evaporated to

near dryness, volume was adjusted to 2 ml with acetone, and aliquots of 0.2 ml were radioassayed in PPO-toluene cocktail. Radioactivity in the "internal aqueous extract" was estimated by counting 0.5-ml aliquots in 10 ml of Bio-fluorTM (New England Nuclear, Boston, MA). ^{14}C content in "unextractable residue" was determined by using the chemical solubilization technique.¹²⁾

Bendiocarb metabolite analysis was conducted using internal organic extracts and container rinses. Container rinses for metabolite analysis were obtained by using 30 SCR. Insect treatment methodology was similar to that of the metabolism study. Holding jars were washed three times with 10 ml of acetonitrile. Extracts were filtered and evaporated, and the final concentrate volume was adjusted to 0.2 ml with acetone. Two 10- μ l aliquots were removed for quantification by LSC. A 50- μ l aliquot of each concentrated sample was cochromatographed with 10 μ l of a solution of nonradioactive bendiocarb and its metabolite standards. Bendiocarb and its metabolites were separated, identified, and estimated by one-dimensional TLC, autoradiography, and LSC as described in the previous TLC section.

5. Penetration Study

Each penetration experiment utilized 10 SCR per replicate, treated with radiolabeled bendiocarb. Treatment methodology was similar to that of the metabolism study. However, only one dosage rate (1/10 LD_{50} value) for adults was used. In addition to the external rinse sets used in the radioactivity distribution section, groups of treated insects were rinsed at shorter time intervals (0, 2, and 10 min posttreatment) to measure the initial penetration rate. Radioactive recovery in the "external rinse" was plotted against time on semilog paper to estimate penetration rate. Each time interval experiment was replicated three times.

RESULTS AND DISCUSSION

1. Toxicity

The topical 48-hr LD_{50} s of bendiocarb to SCR adults and third-instar larvae were 1.3 ± 0.1 and 9.1 ± 4.0 $\mu\text{g/g}$, respectively. Larvae were approximately seven times less susceptible than adults. Similar results were obtained

for laboratory-reared F_1 western corn rootworm, *D. virgifera virgifera* LECONTE, larvae with an LD_{50} value of $7.7 \mu\text{g/g}$.¹³⁾ Bendiocarb phenol did not cause intoxication symptoms at test concentrations as high as $385 \mu\text{g/g}$. Bendiocarb and carbofuran are structurally similar. Their toxicity to SCR adults is identical, whereas bendiocarb is 2.6 times more toxic than carbofuran to SCR larvae.¹⁴⁾

The signs of toxicity produced by bendiocarb were typical fast-acting carbamate intoxication symptoms. After an LD_{50} dosage of $1.3 \mu\text{g/g}$, all beetles were knocked down in 20 min. Recovery began 5 hr after knockdown. Larvae treated at an LD_{50} of $9.1 \mu\text{g/g}$ regurgitated or salivated profusely; knockdown occurred in 10 min, and recovery started in 8 hr.

2. Metabolism and Distribution of Bendiocarb

Distribution of recovered radioactivity in SCR adults and larvae at various times, after topical application with ^{14}C -bendiocarb, are presented in Table 2. Because SCR were knocked down fast by bendiocarb, adults and larvae were treated at $1/10 LD_{50}$ dosage and analyzed at shorter periods after treatment (0.083, 0.5, 1, 2, and 4 hr). At this dosage, however, no knockdown was observed.

The percentage of bendiocarb penetration

was calculated from the difference between total recovery (%) and external rinse (%). SCR larvae showed faster penetration rates than adults during the first 5 min (0.083 hr); approximately 64% of bendiocarb applied to larvae penetrated through the cuticle, whereas, in adults, only 55% of the applied dose penetrated the body. Internal organic extracts represented compounds that were free in the hemolymph or loosely bound to tissues and extractable by organic solvents. The major radioactive portion was found in this fraction.

Radioactive recovery in internal aqueous extracts increased with time. Greater amounts of radioactivity in this fraction from larvae imply that more aqueous soluble metabolites formed in larvae than in adults. Compared with other fractions, only trace amounts of radioactivity were recovered from unextractable residues.

Container rinses of bendiocarb SCR adults and larvae show that, during the first 5 min, larvae excreted in greater amounts and at faster rates than did adults. Between 1 and 2 hr posttreatment, the percentage of excretion was similar between adults and larvae, with recoveries ranging from 12 to 14% of the administered radiocarbon. Two hours after treatment, percentage of excretion increased in

Table 2 Distribution of radioactivity in southern corn rootworms after a topical application of $1/10 LD_{50}$ dosage of ^{14}C -labeled bendiocarb.^{a)}

Stage	Fraction	% applied dose recovered after indicated treatment time (hr)				
		0.083	0.5	1	2	4
Adult ^{b)}	External rinse	44.8	19.8	10.6	8.8	6.0
	Internal organic extract	48.1	64.9	68.3	61.0	52.5
	Internal aqueous extract	0.5	3.8	4.2	7.2	9.1
	Unextractable residue	1.3	2.1	1.3	2.9	2.2
	Container rinse	4.8	10.0	12.7	12.3	20.6
	Total recovery	99.5	100.6	97.1	92.2	90.4
Larva ^{b)}	External rinse	32.8	13.2	11.6	4.7	2.7
	Internal organic extract	50.3	55.3	60.5	64.3	49.1
	Internal aqueous extract	3.4	3.8	6.8	9.0	16.7
	Unextractable residue	.0	.0	0.1	.0	0.2
	Container rinse	10.2	14.9	13.1	13.8	14.4
	Total recovery	96.7	87.2	92.1	91.8	83.1

^{a)} Data represent means of three replicates.

^{b)} $1/10 LD_{50}$ dosage of bendiocarb to adults and larvae are $0.13 \mu\text{g/g}$ and $0.91 \mu\text{g/g}$, respectively.

adults while remaining constant in larvae. Data from internal aqueous extracts and container rinses imply different detoxication mechanisms and/or rates between SCR adults and larvae. SCR larvae also have been shown to excrete isofenphos more rapidly than SCR adults do.¹⁵⁾ A human male volunteer⁶⁾ excreted approximately 36% of the administered dose after oral administration of ¹⁴C-bendiocarb. Male rats, which received single oral administrations of ¹⁴C-bendiocarb at two dosage levels (0.125 and 2.5 mg/kg), excreted approximately 81 and 71% of the administered doses, respectively, within 8 hr posttreatment.⁶⁾ These mammalian data are not directly comparable to the insect data because of differences in route of exposure.

The degradation products of bendiocarb in the internal organic extract and the container rinse are shown in Tables 3 and 4. Bendiocarb metabolites were qualitatively similar but quantitatively different between adults and larvae. Identifiable products were bendiocarb and bendiocarb phenol. Two other radioactive

spots were unknown, and polar metabolites remained at the origin of the chromatogram.

Major metabolites from the internal organic extract differed between SCR adults and larvae (Tables 3 and 4). Bendiocarb phenol was the prominent metabolite in SCR adults, whereas polar metabolites at the chromatogram origin (presumably conjugated compounds) were the dominant metabolites in SCR larvae. One to 4 hr after treatment, more than 36% of bendiocarb applied to SCR adults was recovered as bendiocarb phenol (Table 3). Internal bendiocarb phenol accumulated fast in the first 30 min, going from 4.6% of applied dose at 5 min to 24.5% of applied dose at 30 min. Rapid bendiocarb phenol formation suggests that bendiocarb was transformed primarily to this compound. In SCR larvae, however, bendiocarb phenol was less than 20% of applied dose during the 4-hr experimental period. The major metabolites for SCR larvae were polar metabolites at the chromatogram origin. The amounts increased from 3.5% of the applied dose at 5 min to 37.8% of the applied dose at

Table 3 Nature and amounts of metabolites in internal organic extract and container rinse from southern corn rootworm adults treated with 0.13 μ g/g (1/10 LD₅₀) of ¹⁴C-bendiocarb.

Metabolite	Fraction ^{a)}	% applied dose recovered after indicated treatment time (hr)				
		0.083	0.5	1	2	4
Bendiocarb	I	40.6	35.5	25.3	17.8	11.2
	C	3.1	9.6	11.7	9.8	17.4
	T	43.7	45.1	37.0	27.6	28.6
Bendiocarb phenol	I	4.6	24.5	35.9	37.1	34.8
	C	1.2	0.1	0.4	2.1	2.6
	T	5.8	24.6	36.3	39.2	37.4
Unknown metabolite	I	0.5	0.8	1.4	1.0	1.5
	C	0.2	.0	0.2	0.1	.0
	T	0.7	0.8	1.6	1.1	1.5
Origin	I	2.4	4.0	5.1	4.7	4.8
	C	0.2	0.3	0.3	0.4	0.6
	T	2.6	4.3	5.4	5.1	5.4
Total metabolites	I	7.5	29.3	42.5	42.8	42.1
	C	1.6	0.4	0.9	2.6	3.2
	T	9.1	29.7	43.3	45.4	44.3

^{a)} I: Internal organic extract, C: Container rinse, T: Total amounts from internal organic extract and container rinse.

Table 4 Nature and amounts of metabolites in internal organic extract and container rinse from southern corn rootworm larvae treated with 0.91 $\mu\text{g/g}$ of ^{14}C -bendiocarb.

Metabolite	Fraction ^{a)}	% applied dose recovered after indicated treatment time (hr)				
		0.083	0.5	1	2	4
Bendiocarb	I	39.9	36.8	33.3	19.1	8.7
	C	10.1	10.4	6.9	6.1	9.1
	T	50.0	47.2	40.2	25.2	17.8
Bendiocarb phenol	I	5.9	8.2	7.4	15.2	4.6
	C	0.1	1.8	2.2	2.9	0.9
	T	6.0	10.0	9.6	18.1	5.5
Unknown metabolite	I	1.1	1.3	1.5	2.3	0.7
	C	.0	1.0	1.3	1.7	1.2
	T	1.1	2.3	2.8	4.0	1.9
Origin	I	3.5	9.1	18.4	27.6	35.1
	C	.0	1.2	2.3	3.2	2.7
	T	3.5	10.3	20.7	30.8	37.8
Total metabolites	I	10.5	18.6	27.3	45.1	40.4
	C	0.1	4.0	5.8	7.8	4.8
	T	10.6	22.6	33.1	52.9	45.2

^{a)} I: Internal organic extract, C: Container rinse, T: Total amounts from internal organic extract and container rinse.

4 hr (Table 4).

An isofenphos metabolism study revealed that SCR adults also degrade that organophosphorus (OP) insecticide *via* a hydrolysis pathway to a greater extent than SCR larvae do.¹⁵⁾ Hydrolysis to a phenolic product has been documented in *Diabrotica* for other carbamate¹⁶⁾ and OP¹⁷⁾ insecticides.

With use of TLC in *n*-butanol-acetone-ammonium hydroxide-water (30:10:10:4), components of the chromatogram origin were further separated. Two major radioactive spots were isolated from the chromatogram origin. The *R_f*-values and the ratio of these two isolated components (1:4) were similar between SCR adults and larvae. The results indicate that two conjugated compounds may be formed. In addition, qualitatively similar conjugated compounds imply the same mechanism of conjugation reaction in SCR adults and larvae. Conjugation was studied by acid hydrolysis of the conjugated compounds.⁶⁾ Two and one-half hours after acid hydrolysis,

some radioactivity was found at the spot corresponding to bendiocarb phenol. These results suggest that the chromatogram origin included conjugates of bendiocarb phenol. Additional support for this hypothesis was the marked disappearance of bendiocarb phenol, which corresponded to the rapid increase of the chromatogram origin between 2 and 4 hr after treatment in SCR larvae (Table 4). Because enzymatic hydrolysis of conjugated compounds was not conducted in the present experiment, endogenous substances used for conjugation reactions were unidentified. Metabolism of bendiocarb in the rat and man, however, indicated that major conjugated materials were sulfate and glucuronide conjugates of bendiocarb phenol.⁶⁾ These authors also found small amounts of conjugates of 2,2-dimethylbenzo-1,3-dioxol-4-yl *N*-(hydroxymethyl)carbamate at early metabolic stages in the rat and man. In summation, the suggested major metabolic pathway of bendiocarb in SCR adults and larvae was hydrolysis of the

carbamate ester bond to yield bendiocarb phenol and the subsequent conjugation of bendiocarb phenol.

Although SCR adults and larvae exhibit similar decreases in internal bendiocarb (Tables 3 and 4) and a common major metabolic pathway, different major metabolites suggest that SCR adults, when compared with larvae, detoxify bendiocarb by using a similar mechanism but differ in having slow conjugation reactions. In SCR adults during the first hour, most of the bendiocarb was rapidly hydrolyzed into noninsecticidal bendiocarb phenol. After 1 hr, the amount of bendiocarb phenol remained constant throughout the 4 hr experimental time. Little variation of bendiocarb phenol 1 hr after treatment and low recovery of ^{14}C label at the chromatogram origin indicate that the conjugation reaction occurred only quite slowly in SCR adults. In SCR larvae, however, the conjugation reaction followed the hydrolysis reaction immediately. A prominent increase of conjugated compounds at the chromatogram origin with time and slow accumulation of bendiocarb phenol during the first 2 hr imply that bendiocarb phenol was metabolized rapidly into conjugated compounds. In addition, the apparent decrease of bendiocarb phenol at 4 hr posttreatment may have resulted from smaller reserves of internal bendiocarb and subsequent conjugation of bendiocarb phenol.

Excretion of parent bendiocarb was another detoxication mechanism utilized by SCR adults, whereas excretion of bendiocarb by SCR larvae was less important. In summation, SCR adults detoxified bendiocarb primarily by hydrolyzing it rapidly into nontoxic bendiocarb phenol and secondarily by excreting the toxic parent compound. SCR larvae, however, detoxified bendiocarb primarily by forming conjugated compounds.

Past literature^{11,18)} reported that large amounts of applied dosage will change the penetration rate, rate of metabolism and excretion rate of the insecticide. Bendiocarb applied at a greater dosage (LD_{10}) to SCR adults revealed an obvious effect on the hydrolytic enzyme system (Fig. 2). A lower percentage of bendiocarb phenol and a higher percentage of internal bendiocarb suggested that more time

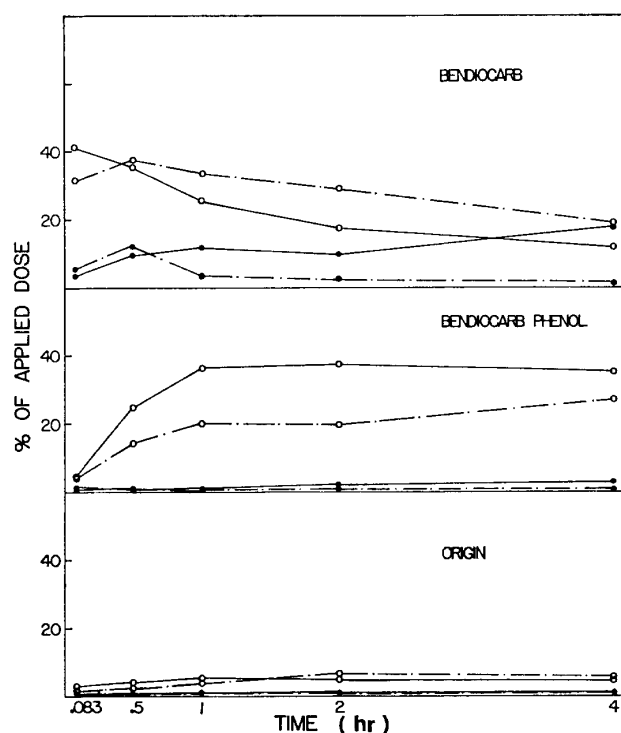


Fig. 2 Recovery of bendiocarb, bendiocarb phenol, and metabolites at origin in internal organic extract (○) and container rinse (●) from southern corn rootworm adults treated at two dosage levels, 0.13 $\mu\text{g/g}$ (solid lines) and 0.80 $\mu\text{g/g}$ (dot-dashed lines).

was needed to accomplish the same percentage of hydrolysis. Bendiocarb phenol represented 27.3% of the applied dose 4 hr after treatment at the larger dose, indicating that the hydrolytic reaction was not complete at 4 hr. Because the enzyme kinetics were not studied in the present experiment, saturation of hydrolytic enzyme at the higher dose was uncertain. Possible saturation kinetics at a higher dose has been reported in house flies treated with carbofuran,¹⁸⁾ methyl parathion, and fenitrothion.¹¹⁾

3. Penetration Rate

Penetration of ^{14}C -bendiocarb into SCR adults and larvae was measured from the amounts of ^{14}C recovered in external rinses at predetermined intervals. Results of bendiocarb penetration are shown in Fig. 3. The penetration curve was biphasic for adults and triphasic for larvae. Each curve exhibited a rapid initial penetration rate (phase I), followed by slow, steady-state rates of decline

(phase II and phase III). The kinetics in each phase is explained in detail by Elliott,¹⁹⁾ Lewis,²⁰⁾ and Brooks.²¹⁾ According to Elliott,¹⁹⁾ penetration rate is measured during the phase II of the penetration curve. Rate constants of penetration (k_p) were expressed by

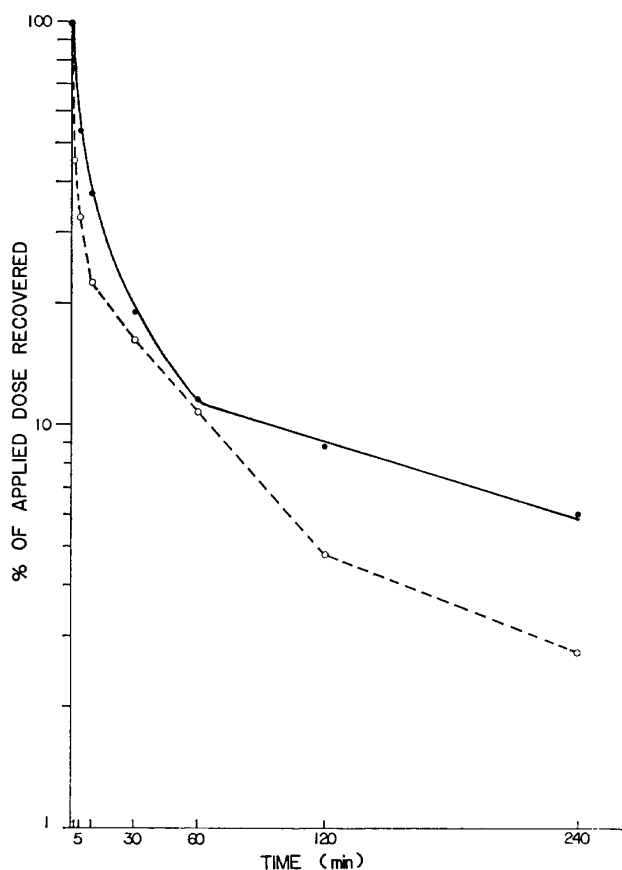


Fig. 3 Penetration of bendiocarb into southern corn rootworms after topical application of ^{14}C -bendiocarb at 1/10 LD_{50} dosages ($0.13 \mu\text{g/g}$ for adults (●) and $0.91 \mu\text{g/g}$ for larvae (○)).

Penetration curves were obtained by plotting the logarithm of percentage recovery of ^{14}C in external rinse versus time.

the slope of the straight line during the second phase. For bendiocarb, rate constants were nearly 4.2 times larger for larvae ($k_p = 0.0140 \text{ min}^{-1}$) than for adults ($k_p = 0.0033 \text{ min}^{-1}$). Cuticular permeability²⁰⁾ and protein and lipid content of the cuticle²²⁾ may influence insecticidal penetration rate. The faster penetration rate in larvae implies that penetration mechanism probably was unimportant to selective toxicity of bendiocarb between SCR adults and larvae.

4. Amounts of Internal Toxic Compounds

Insecticide toxicity depends upon penetration rate, rate of metabolism, and excretion rate of the insecticide, and, for OP and carbamate insecticides, acetylcholinesterase (AChE) sensitivity to insecticide inhibition.^{23,24)} The first three factors determine the amount and duration of insecticidal compounds within the body.²⁵⁾ Because parent bendiocarb was the only compound toxic to SCR adults and larvae in toxicity tests, amounts of the internal toxic dose were estimated solely on bendiocarb recovery from internal organic extracts. Table 5 shows that the adult/larva ratio of internal bendiocarb (mean of 0.14 for 0.083, 0.5, 1, 2, 4 hr) was similar to the adult/larva ratio of the applied dose (0.14). These results suggest that the amount of internal toxic compound was not a determining factor for the sevenfold toxicity difference between SCR adults and larvae. Comparable ratios for internal bendiocarb and applied doses may be attributed to similar combined effects of penetration rate, rate of metabolism, and excretion rate of bendiocarb between adults and larvae. Differences in AChE sensitivity to determining

Table 5 Amounts ($\mu\text{g/g}$) of internal toxic compound^{a)} recovered in southern corn rootworms after topical application of 1/10 LD_{50} dosage of ^{14}C -bendiocarb and ratios between adults and larvae.

	Dose applied ($\mu\text{g/g}$)	Time (hr)				
		0.083	0.5	1	2	4
Adult	0.13	0.053	0.046	0.033	0.023	0.015
Larva	0.91	0.363	0.335	0.303	0.174	0.079
Adult/larva ratio	0.14	0.15	0.14	0.11	0.13	0.19

^{a)} Amounts of parent bendiocarb from internal organic extract.

bendiocarb effects on SCR would be necessary to confirm this possibility.

ACKNOWLEDGMENTS

The authors are grateful for financial support provided by the U.S. Department of Agriculture, Brookings, South Dakota, through Cooperative Agreement No. 38-519B-0-0923. We thank Dr. Paul Dahm for his role in the initiation of this project. This is Journal Paper No. J-11755 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 2306.

REFERENCES

- 1) Fisons Corporation: "NC 6897—Experimental Insecticide (technical information bulletin)," Fisons Corporation, Bedford, 1979
- 2) T. L. Ladd, Jr., K. O. Lawrence & M. G. Klein: *J. Econ. Entomol.* **76**, 551 (1983)
- 3) G. Barson & N. G. McCheyne: *Ann. Appl. Biol.* **90**, 147 (1978)
- 4) N. Eshghy, B. Janbakhsh & M. Motabar: *Mosquito News* **39**, 126 (1979)
- 5) S. Y. Szeto, A. T. S. Wilkinson & M. J. Brown: *J. Agric. Food Chem.* **32**, 78 (1984)
- 6) I. R. Challis & J. W. Adcock: *Pestic. Sci.* **12**, 638 (1981)
- 7) Anonymous: *Bull. Entomol. Soc. Am.* **18**, 179 (1972)
- 8) T. F. Branson, P. L. Guss, J. L. Krysan & G. R. Sutter: U.S. Dep. Agric., Agric. Res. Serv. (Publ.) NC 28, p. 18, 1975
- 9) C. I. Bliss: *Ann. Appl. Biol.* **22**, 134 (1935)
- 10) L. C. Miller & M. L. Tainter: *Proc. Soc. Exp. Biol. Med.* **57**, 261 (1944)
- 11) R. M. Hollingworth, R. L. Metcalf & T. R. Fukuto: *J. Agric. Food Chem.* **15**, 250 (1967)
- 12) D. T. Mahin & R. T. Lofberg: *Anal. Biochem.* **16**, 500 (1966)
- 13) D. D. Walegenbach & G. R. Sutter: Proc. 29th Ill. Custom Spray Oper. Train. Sch., University of Illinois, Urbana, pp. 68–71, 1977
- 14) B. A. Solheim: Ph. D. dissertation, University Microfilms Order No. 82-24334, Iowa State University, Ames, p. 104, 1982
- 15) C.-Y. Hsin & J. R. Coats: *Pestic. Biochem. Physiol.* **25**, 336 (1986)
- 16) H. Chio & R. L. Metcalf: *J. Econ. Entomol.* **72**, 732 (1979)
- 17) C. C. Conaway & C. O. Knowles: *J. Econ. Entomol.* **62**, 286 (1969)
- 18) C. Collins, J. M. Kennedy & T. Miller: *Pestic. Biochem. Physiol.* **20**, 25 (1983)
- 19) M. Elliott, M. G. Ford & N. F. Janes: *Pestic. Sci.* **1**, 220 (1970)
- 20) C. T. Lewis: *J. Insect Physiol.* **11**, 683 (1965)
- 21) G. T. Brooks: "Insecticide Biochemistry and Physiology," ed. by C. F. Wilkinson, Plenum Press, New York, pp. 3–58, 1976
- 22) S. B. Vinson & P. K. Law: *J. Econ. Entomol.* **64**, 1387 (1971)
- 23) R. J. Kuhr: *J. Agric. Food Chem.* **18**, 1023 (1970)
- 24) R. K. Tripathi & R. D. O'Brien: *Pestic. Biochem. Physiol.* **3**, 495 (1973)
- 25) A. P. Kulkarni & E. Hodgson: "Introduction to Biochemical Toxicology," ed. by E. Hodgson & F. E. Guthrie, Elsevier, New York, pp. 106–132, 1980

要 約

Diabrotica undecimpunctata howardi (ハムシの一種)の成虫および幼虫における bendiocarb の代謝

Chu-Ying Hsin, Joel R. Coats

Bendiocarb は標的昆虫の幼虫に対し、成虫より7倍毒性が低い。LD₅₀はそれぞれ9.1および1.3 μg/gである。¹⁴C-Bendiocarbを、幼虫・成虫のそれぞれに各LD₅₀の1/10量点滴投与し代謝物を調べたところ、前者では抱合代謝物が、後者では bendiocarb phenol が主代謝物であった。主代謝経路は、エステル加水分解、ついで生じたフェノールの抱合というものである。上記と同様の投与量において幼虫と成虫の体内における親化合物残存量の経時変化を調べたところ、幼虫体内残存量と成虫体内残存量の比はだいたい一定であり、体内残存量の差異が幼虫成虫間の選択毒性の原因ではないことがわかった。