Detection and Quantification of Trace Levels of Carbaryl and Its Metabolites from *Dendroctonus frontalis* Zimmermann

He Zhong,† Felton L. Hastings,* and Fred P. Hain

Department of Entomology, North Carolina State University, Raleigh, North Carolina 27695-7626

A direct and sensitive high-performance liquid chromatographic (HPLC) method was developed for the detection and quantitative analysis of carbaryl and metabolites at the parts-per-billion level using a YMC stainless steel reversed-phase ODS (μBondapak C_{18}) HPLC column (250 mm × 3.9 nm i.d.), equipped with a Waters Guard-Pak C_{18} precolumn module. The use of this methodology combined with radioisotopic technique is demonstrated by topically treating the southern pine beetle, *Dendroctonus frontalis* Zimmermann, with [*naphthyl*-1-^{14}C]carbaryl and analyzing methanolic extracts of the exoskeleton, the vials in which beetles were held, and the whole body homogenates at various incubation time intervals after treatment. All extracts were passed through a Millex-HV 0.45-μm filter unit (Millipore, Bedford, MA) prior to HPLC analysis. Advantages of this method include high sensitivity, reproducibility, short analysis time, and good recovery. This HPLC method should be useful for toxicological and environmental studies that require the detection of nanogram concentrations of carbaryl and carbaryl metabolites.

INTRODUCTION

Carbaryl (1-naphthyl *N*-methylcarbamate) is a broad spectrum insecticide currently approved by the EPA at 2% active ingredient for the control of bark beetles. It is effective against western species of bark beetles (Hall et al., 1982; Page et al., 1985; Werner et al., 1986) but was not effective in protecting trees from epidemic outbreaks of southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae), in field tests (Berisford et al., 1981). The elucidation of penetration, excretion, and internal transformation processes of carbaryl within the beetle should shed light on this lack of efficacy.

Numerous high-performance liquid chromatographic (HPLC) methods have been reported for the determination of carbaryl and its oxidative product, 1-naphthol, but the other metabolites of carbaryl were not quantified simultaneously by this technique (Spittler et al., 1986; Ward et al., 1987; Brayan et al., 1988; Kawai et al., 1988). Strait et al. (1991) reported a sensitive HPLC procedure for detecting carbaryl, 1-naphthol, and methylolcarbaryl in pond water and serum using a 3-μm C_{18} Ultrasphere column. Although our column and conditions were different, we experienced additional problems of trace amounts of carbaryl metabolites cochromatographing with extraneous material from the beetle. By combining HPLC methodology with radiolabeled techniques, this problem was substantially reduced.

This paper presents a simple extraction and filtration process followed by HPLC analysis on a highly efficient YMC stainless steel reversed-phase ODS (μBondapak C_{18}) HPLC column incorporated with radioisotopic technique for the simultaneous quantitative separation and determination of carbaryl and its metabolites within the nanogram range. The procedure proved useful for the detection of carbaryl on the beetle exoskeleton, within whole body homogenates, and within the incubation chamber. Therefore, it may be suitable for use in insect metabolism, environmental degradation, and toxicokinetics studies.

† Present address: Harbor Branch Oceanographic Institution, 5600 U. S. 1 North, Fort Pierce, FL 34946.

MATERIALS AND METHODS

Instrumentation and Chromatography. The methods were developed on a Waters high-performance liquid chromatography system, which consisted of Millipore Waters (Milford, MA) Model 510 pumps, a Model 490E programmable multiwavelength UV detector at 217 nm, a U6K universal liquid chromatograph injector, a Maxima 820 chromatography workstation with Maxima 820 version 3.0 software, and an NEC 286 PowerMate 2 with an EGA color monitor and a system interface module (SIM). The column was a YMC stainless steel reversed-phase ODS (μBondapak C_{18}) HPLC column (250 mm × 3.9 μm i.d.), equipped with a Waters Guard-Pak C_{18} precolumn module. Radioactivity was determined with a 5801 liquid scintillation counter equipped with an EPSON printer (Beckman Instruments, Irvine, CA). Centrifugation was with a Sorvall RC2-B superspeed automatic refrigerated centrifuge.

Elution was carried out with methanol/water, (55:45) at a flow rate of 1.2 mL/min. The total time to complete the chromatographic analysis of each sample was 20 min. Standard curves were prepared by using serial dilutions of a stock solution (100 μg/mL) and 5 μL was applied to the HPLC column (0.05–400 ng/5 μL injection range). Analytical grade carbofuran was used as an internal standard to calculate and quantify the concentration of carbaryl and its metabolites.

Reagents. Carbaryl (1-naphthyl methylcarbamate), carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), 1-naphthol, methylolcarbaryl (1-naphthyl hydroxymethyl carbamate), demethylcarbaryl (1-naphthyl carbamate), 4-hydroxycarbaryl (4-hydroxy-1-naphthyl methylcarbamate), and 5-hydroxycarbaryl (5-hydroxy-1-naphthyl methylcarbamate) were gifts from Dr. Nathan Andrawes, Rhône-Poulenc Agricultural Co., Research Triangle Park, NC. [*naphthyl*-1-^{14}C]Carbaryl (purity, >98%; specific activity, 8.5 mCi/mmol; from Sigma), 1,4-naphthalenediol (1,4-dihydroxynaphthalene), and 1,5-naphthalenediol (1,5-dihydroxynaphthalene) were gifts from Dr. Walter Dauterman, Department of Toxicology, North Carolina State University, Raleigh, NC (Figure 1). The solvents used in this work were HPLC grade methanol (MeOH) and acetone (Fisher, Pittsburgh, PA). A Millex-HV 0.45-μm filter unit (Millipore, Bedford, MA) was used for the cleanup of crude beetle extracts used in this study.

Although carbaryl could be quantified on the exoskeleton and within tissue homogenates by HPLC methodology, we were not able to quantify the metabolites which were produced in trace amounts. Adding to the complexity was that some of the metabolites coeluted with extraneous peaks from beetles. There-

0021-8561/94/1442-0949$04.50/0

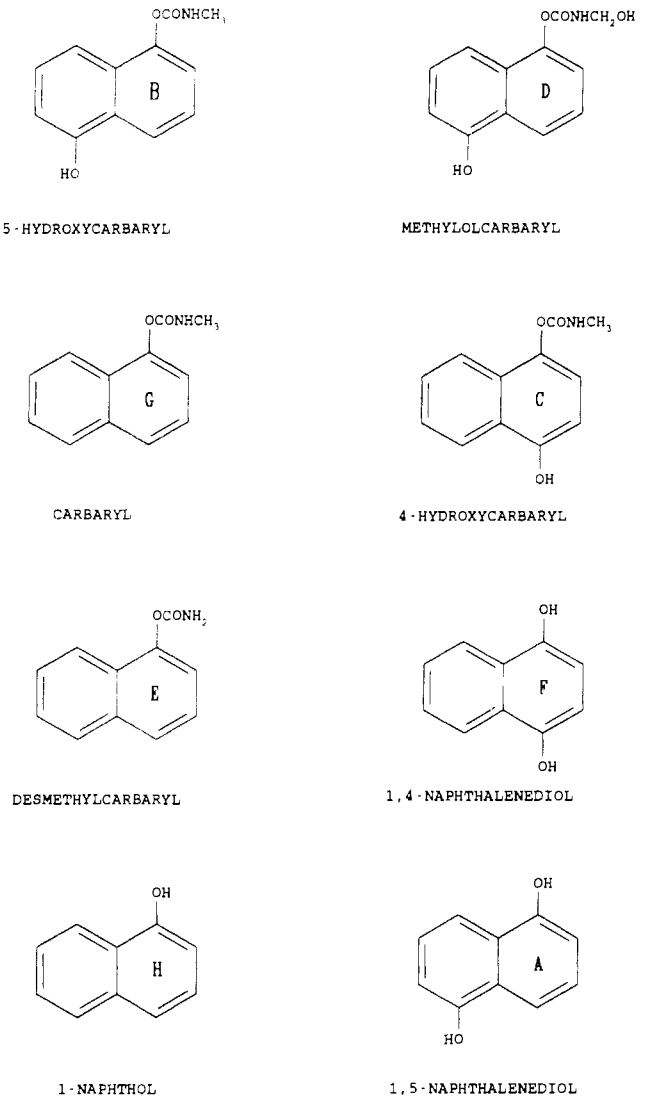

Figure 1. Structure of carbaryl (G) and its potential metabolites: 1,5-naphthalenediol (A); 5-hydroxycarbaryl (B); 4-hydroxycarbaryl (C); methylolcarbaryl (D); demethylcarbaryl (E); 1,4-naphthalenediol (F); 1-naphthol (H).

fore, we incorporated radioisotopic quantification with our HPLC methodology.

Topical Application. The southern pine beetles were obtained from a beetle colony collected from North Carolina and maintained on freshly cut pine bolts. [*naphthyl*-^{14}C]Carbaryl was freshly prepared in HPLC grade acetone. A 0.5-μL droplet was topically applied to the dorsal prothorax area of each adult beetle with a 25-μL Hamilton syringe equipped with a PB600 dispenser (Hamilton). The amount of 1250 dpm/0.5 μL (equivalent to 13 ng of carbaryl/0.5 μL) was applied to each beetle.

Treatments were grouped according to incubation times; these were zero time and 0.5 and 1 h. Three replicates of 20 beetles each were used for the zero time incubation; for other incubation times, six replicates of 20 beetles each were used. Sixty beetles served as controls; these were treated with 0.5 μL of acetone only. The average beetle body weight was 1.94 mg. All beetles were treated less than 4 h after emergence and were actively moving inside the collection jar.

A 20-mL liquid scintillation glass vial was used as an incubation chamber for both control and treatment beetles. The temperature of incubation was 22 ± 1 °C, and the relative humidity was 80%.

After the beetles were removed from the holding vial, they were washed with 1 mL of MeOH for 5 min on a wrist action shaker (Burrell) and then the MeOH was removed and placed in a clean vial; the beetles were rinsed twice more with 1 mL of MeOH. The 3 mL of combined MeOH wash was passed through

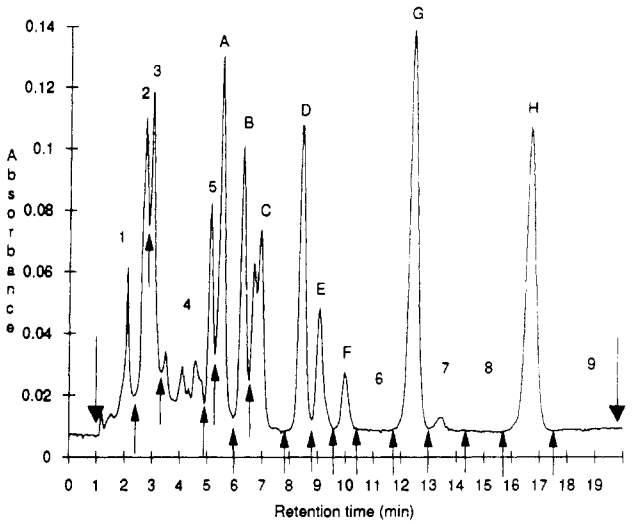

Figure 2. Fractionation of [^{14}C]carbaryl and metabolites extracted from exoskeleton of beetles. Peaks A–H were standards added (refer to Figure 1 for compound names) and cochromatographed with the radioactivity for peak collection purposes; the unknowns 1–9 were extraneous peaks and nonpeak areas. Each region of fraction collection was between the arrows.

a 0.45-μm filter unit and taken to dryness under a hood by a gentle nitrogen stream and the particulate picked up in 100 μL of MeOH.

The material remaining in the incubation vessels was removed by adding 1 mL of MeOH and sonicating (Disintegrator System Eighty, Ultrasonic) for 5 min. Each vial was rinsed three times and combined to one vial for each treatment group. The extracts were filtered, evaporated to dryness, and resuspended in 100 μL of MeOH.

The internal body tissue was extracted by adding 2 mL of MeOH per 30 beetles and homogenizing (Polytron, Brinkmann Instruments, Westbury, NY) for 2 min to break down the beetle's hard exoskeleton. The homogenizer tip was washed with 2 mL of MeOH after each homogenate, giving a total volume of 4 mL. The extracts were centrifuged for 15 min at 1250*g* at 0 °C. The supernatant was decanted and the pellet resuspended in 2 mL of MeOH followed by 10 min on a wrist action shaker (Burrell) and recentrifuged. The procedure was repeated four times for each sample. The combined supernatant extracts were filtered and taken to dryness under a hood by a gentle nitrogen stream and resuspended in 100 μL of MeOH. To collect carbaryl and trace amount of metabolites at correct retention times, 100-μL standard solutions were added to all extracts.

The HPLC was calibrated by standard solutions each day. Ten microliters of insect extracts was injected into and chromatographed on HPLC. The elution solvent was collected in 20-mL liquid scintillation vials, and 7–10 collections were combined to have sufficient radioactivity for reliable counts. There were 16 collection regions including standard carbaryl, and its potential metabolites, extraneous peaks, and nonpeak areas (Figure 2). The collection vials were placed under the hood, and the MeOH was removed by using a gentle nitrogen stream. Ten milliliters of OPTI-FLUOR cocktail (Packard Instrument Co., Downers Grove, IL) was added to each scintillation vial, and the vials were counted by liquid scintillation for direct measurement of the radioactivity in each collection area. Identification was based on cochromatography using known standards.

RESULTS

Standard Curves for Carbaryl and Metabolites. The capacity factor k' value and selectivity α value of carbaryl and its metabolites are shown in Table 1. All k' values are between 2 and 10, and all α values are larger than 1. The critical pairs have a separation ratio (α) of 1.10. The detection limit of this HPLC method was about 10 ng/mL; each compound was clearly separated by the conditions of analysis, and all compounds were eluted from

Table 1. Elution Parameters[a] of Carbaryl and Its Metabolites

code	name	retention t'_r (min)	k'	α
A	1,5-naphthalenediol	5.50	2.07	1.15
B	5-hydroxycarbaryl	6.33	2.52	1.22
C	4-hydroxycarbaryl	7.05	2.92	1.16
D	methylolcarbaryl	8.48	3.71	1.27
E	demethylcarbaryl	9.13	4.07	1.10
F	1,4-naphthalenediol	10.21	4.67	1.15
G	carbaryl	12.58	5.99	1.28
H	1-naphthol	17.04	8.47	1.41

[a] k' is capacity factor [$k' = (t_r - t_0)/t_0$], α is selectivity [$\alpha = k'_B/k'_A$], and t_r is retention time. Calculation was based on t_0 = 1.8 min. Ideal capacity factor k' = 2–10, and selectivity $\alpha > 1$.

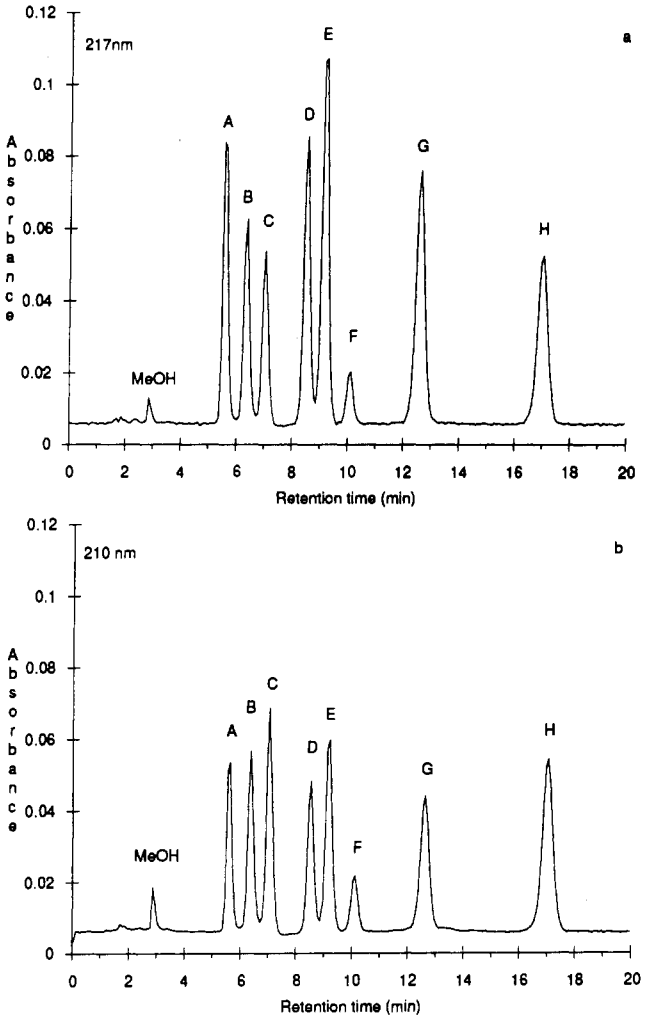

Figure 3. Reversed-phase HPLC chromatogram of standard carbaryl and its metabolites viewed by multiwavelength UV detector. The compound peaks were responses to 10 ng/5 μL injections at 217 (a) and 210 nm (b). Most compounds were less sensitive to 210 nm than to 217 nm. The legend letters correspond to the letters in Figure 1.

the column in less than 18 min (Figure 3a). Selection of the best chromatographic UV-absorbing wavelength and solvents is vital to the HPLC technique's sensitivity, selectivity, efficiency, and resolution. For this experiment 217 nm was more sensitive to carbaryl and its metabolites than 210 nm (Ward et al., 1987; Strait et al., 1991) (Figure 3b), 225 nm, 245 nm (Brayan et al., 1988), 267 nm (Kawai et al., 1988), 280 nm (Jones et al., 1982), 235 nm, and 254 nm (product information, Sigma Chemical Co., St. Louis, MO) in general (Table 4), and methanol/water gave better resolution than acetonitrile/water as a mobile phase (Jones et al., 1982).

Table 2. Fate of [*naphthyl*-^{14}C]Carbaryl following Topical Application on Southern Pine Beetle Adult Prothorax after Incubation for 0.5 h at 22 °C[a]

		% relative recovery of radioactivity			
metabolite	fraction (min)	exo-skeleton	internal	vial	total
unknown 1	0.5–2.2	0.47	0.01	0.49	0.97
unknown 2	2.2–2.6	1.41	0.32	1.29	3.02
unknown 3	2.6–3.8	0.61	0.32	0.49	1.42
unknown 4	3.8–4.7	0.71	0.22	0.43	1.36
unknown 5	4.7–5.4	0.28	0.07	0.18	0.53
1,5-naphthalenediol	5.4–6.1	0.26	0.10	0.17	0.53
5-hydroxycarbaryl	6.1–6.6	0.33	0.05	0.14	0.52
4-hydroxycarbaryl	6.6–7.6	0.55	0.13	0.16	0.84
methylolcarbaryl	7.6–8.9	0.67	2.75	0.18	3.60
demethylcarbaryl	8.9–9.9	0.24	0.14	0.10	0.48
1,4-naphthalenediol	9.9–12.1	0.59	0.60	0.21	1.40
carbaryl	12.1–13.3	63.73	16.26	3.09	83.08
unknown 6	13.3–14.6	0.25	0.06	0.25	0.56
unknown 7	14.6–16.8	0.24	0.02	0.08	0.34
1-naphthol	16.8–17.7	0.68	0.22	0.17	1.07
unknown 8	17.7–20.0	0.20	0.01	0.07	0.28
total		71.22	21.28	7.50	100.0

[a] The total actual recovery of applied dose was 92.47% after a 0.5-h incubation. For convenience of comparing the relative percentage of each compound, this table is based on 100% recovery. The absolute percent recovery ratio can be obtained by ($X \times 92.47$)/100, for example: internal percent carbaryl recovery (16.26 × 92.47)/100 = 15.04.

Table 3. Fate of [*naphthyl*-^{14}C]Carbaryl following Topical Application on Southern Pine Beetle Adult Prothorax after Incubation for 1 h at 22 °C[a]

		% relative recovery of radioactivity			
metabolite	fraction (min)	exo-skeleton	internal	vial	total
unknown 1	0.5–2.2	0.13	0.0	0.40	0.53
unknown 2	2.2–2.6	0.48	0.91	1.72	3.11
unknown 3	2.6–3.8	0.20	0.83	0.89	1.92
unknown 4	3.8–4.7	0.39	0.44	0.34	1.17
unknown 5	4.7–5.4	0.04	0.04	0.15	0.23
1,5-naphthalenediol	5.4–6.1	0.10	0.03	0.14	0.27
5-hydroxycarbaryl	6.1–6.6	0.08	0.10	0.11	0.29
4-hydroxycarbaryl	6.6–7.6	0.25	0.27	0.20	0.72
methylolcarbaryl	7.6–8.9	0.43	3.80	0.14	4.37
demethylcarbaryl	8.9–9.9	0.09	0.05	0.09	0.23
1,4-naphthalenediol	9.9–12.1	0.73	0.25	0.15	1.13
carbaryl	12.1–13.3	63.74	20.36	0.28	84.38
unknown 6	13.3–14.6	0.20	0.07	0.08	0.35
unknown 7	14.6–16.8	0.10	0.04	0.07	0.21
1-naphthol	16.8–17.7	0.48	0.32	0.08	0.88
unknown 8	17.7–20	0.05	0.03	0.11	0.19
total		67.52	27.54	4.94	100.0

[a] The total actual recovery of applied dose was 89.83% in 1-h incubation. For convenience of comparing the relative percentage of each compound, this table is based on 100% recovery. The absolute percent recovery ratio can be obtained by ($X \times 89.83$)/100, for example: internal percent carbaryl recovery (20.36 × 89.83)/100 = 18.29.

Total Relative Radioactivity Recovery. All replicates were pooled due to the trace amount of metabolites produced. We recovered 83.08% (in 0.5-h incubation time, Table 2) and 84.38% (in 1-h incubation time, Table 3) of the radiolabeled carbaryl from the beetles' exoskeleton, internal tissues, and holding vials. Methylolcarbaryl (3.60%, 0.5 h; 4.37%, 1 h) was a major oxidative product, and both 4-hydroxycarbaryl (0.84%, 0.5 h; 0.72%, 1 h) and 5-hydroxycarbaryl (0.52%, 0.5 h; 0.29%, 1 h) were present in detectable amounts. The hydrolytic metabolites occurred in the following order: 1,4-naphthalenediol (1.40%, 0.5 h; 1.13%, 1 h) > 1-naphthol (1.07%, 0.5 h;

Table 4. Percentage of UV Wavelength Sensitivity of Carbaryl and Metabolites Compared to the Sensitivity at 217 nm

	% 217 nm of UV wavelength					
	210 (nm)	225 (nm)	245 (nm)	254 (nm)	267 (nm)	280 (nm)
1,5-naphthalenediol	60.7	165.5	11.9	10.7	13.1	21.1
5-hydroxycarbaryl	90.5	107.9	14.3	12.7	14.3	20.6
4-hydroxycarbaryl	125.9	77.7	24.1	14.8	16.7	22.2
methylolcarbaryl	56.5	100.0	10.6	10.6	15.3	15.3
demethylcarbaryl	56.1	99.1	8.4	9.3	13.1	14.0
1,4-naphthalenediol	108.9	108.9	227.7	207.9	69.3	34.6
carbaryl	57.9	101.3	10.5	11.8	15.8	17.1
1-naphthol	103.8	84.6	15.4	15.4	17.3	19.2

0.88%, 1 h) > 1,5-naphthalenediol (0.53% 0.5 h; 0.27%, 1 h). The unknowns **1–5** were numbered according to the order of elution time. The sum totals of their radioactivity were 7.30% at 0.5 h and 6.98% at 1 h. These unknowns may correspond to the water-soluble products of previous studies (Dorough and Casida, 1964; Andrawes and Dorough, 1967). The retention times of unknowns **6** and 7 were between those of carbaryl and 1-naphthol. The total radioactivities were 0.90% (0.5 h) and 0.56% (1 h). Unknown 8 was collected after 1-naphthol and was less than 0.3% of the radioactivity recovered for both incubation times. The amount of unextractable radiolabeled material was not included in this study.

Exoskeleton Wash. Carbaryl and its trace metabolites were detected on the beetle exoskeleton at 0.5 and 1 h after topical application. The average amounts of carbaryl remaining on the exoskeleton were 63.73% (0.5 h, Table 2) and 63.74% (1 h, Table 3). In 0.5-h incubation, the sum total of unknown metabolites **1–5** was 3.48%; the percentage of the major metabolites was in the order 1-naphthol (0.68%) > methylolcarbaryl (0.67%) > 1,4-naphthalenediol (0.59%) > 4-hydroxycarbaryl (0.55%), and the other metabolites produced were less than 0.4% (Table 2). The sum of unknown metabolites (**1–5**) in 1-h incubation was 1.26%, and all other metabolites were less than 0.5% with the exception of 1,4-naphthalenediol, which was 0.73% (Table 3).

Internal Wash. The average total amounts of radioactivity recovered from the internal tissue were 21.28% (0.5 h, Table 2) and 27.54% (1 h, Table 3). Unchanged carbaryl was found in amounts of 16.26% and 20.36% for the 0.5- and 1-h incubation times, respectively. One major oxidative metabolite, which was tentatively identified as methylolcarbaryl, accounted for 2.75% (0.5 h) and 3.80% (1 h) of the radioactivity. The other oxidative metabolites coeluted with 4-hydroxycarbaryl (0.13%, 0.5 h; 0.27%, 1 h) and 5-hydroxycarbaryl (0.05%, 0.5 h; 0.10%, 1 h). Two hydrolytic metabolites were tentatively identified as 1-naphthol (0.22%, 0.5 h; 0.32%, 1 h) and 1,4-naphthalenediol (0.60%, 0.5 h; 0.25%, 1 h). The major unknowns **2** (0.32%, 0.5 h; 0.91%, 1 h), **3** (0.32%, 0.5 h; 0.83%, 1 h), and **4** (0.22% 0.5 h; 0.44%, 1 h) were also found within the beetle.

Vial Wash. Carbaryl and its metabolites were also detected inside the holding vials in both the 0.5- (Table 2) and 1-h (Table 3) incubation times. Because the amounts were minuscule, we combined all extracts of each replicate. The strong hydrophilic unknown compounds 1–4 accounted for about 2.70% (0.5 h) and 3.35% (1 h) of the applied radioisotope, which was about 36% (0.5 h) and 68% (1 h) of the total radioactivity [e.g., in 1 h (3.35%/4.94% × 100 = 68%); Table 3]. The metabolites (and percentages) tentatively identified were 4-hydroxycarbaryl (0.16%, 0.5 h; 0.20%, 1 h), methylolcarbaryl (0.18%, 0.5 h; 0.14%, 1 h), 1,5-naphthalenediol (0.17%, 0.5 h; 0.14%, 1 h), 5-hydroxycarbaryl (0.14%, 0.5 h; 0.11%, 1 h), demethylcarbaryl (0.1%, 0.5 h; 0.09%, 1 h), 1-naphthol (0.17%, 0.5 h; 0.08%, 1 h), and 1,4-naphthalenediol (0.21%, 0.5 h; 0.15%, 1 h). About 3.09% of the carbaryl was contained in the 0.5-h holding vials, and at 1 h this value was 0.28%. This carbaryl probably resulted from mechanical removal as the beetles came in contact with the vials. Andrawes and Dorough (1967) reported similar findings with boll weevils.

DISCUSSION

Although our HPLC technique was sensitive for separation and detection of carbaryl and its metabolites, some metabolites coeluted with extraneous material from beetles which made the separation and identification process impossible. Besides, the trace amounts of carbaryl metabolites were easily lost during the sample cleanup. We therefore incorporated radioisotope techniques with HPLC separation to simplify the sample handling process. This allowed visualization of all standard peaks and the collection of peaks corresponding to known standards. The results confirmed in part our earlier HPLC findings which indicated that 55–65% of the carbaryl remained on the exoskeleton, 2–6% was in the reaction vials, and 13–18% was within the tissue in the 1-h incubation. The amounts of carbaryl determined by radioisotope were 64%, 3%, and 16% in the 0.5-h exposure and 64%, 0.3%, and 20% on the exoskeleton, in the vials, and within the tissue in the 1-h exposure. Obviously, the amount estimated within the vials was not within the detectable limits of the spectrophotometric HPLC analysis, while the estimates for the exoskeleton and within the tissue were in good agreement. The advantages of our combining the radioisotopic technique with HPLC are to extract the metabolite peaks which cochromatograph with extraneous compounds, to trace the origin of the new peaks and to reduce the number of cleanup steps for biological samples, thus avoiding the loss of compound. It also lowers the detection limit and increases the accuracy of identification by HPLC.

Carbaryl and its potential metabolites are all methanol soluble (Figure 2), and three or four extractions with MeOH remove over 90% of the parent compound and degradation products. The total actual recovery of applied carbaryl was 92.47% at 0.5 h (Table 2 footnote) and 89.83% at 1 h (Table 3 footnote). The initial penetration of carbaryl into the beetle (18.29%, Table 3 footnote) was faster than that of boll weevil (5.5%; Andrawes and Dorough, 1967). The metabolism of carbaryl by the beetle is a complex process which produced many intermediate oxidative and hydrolytic products similar to other insects (Dorough and Casida, 1964; Andrawes and Dorough, 1967; Kuhr, 1970; Schlagbauer and Schlagbauer, 1972). Methylolcarbaryl was a major internal metabolite; this was also reported as a major metabolite of the chicken (Strait et al., 1991). The results from the 0.5-h exposure time (Table 2) are surprisingly matched with those of 1 h (Table 3) with the exception of the amount of carbaryl found in the incubation vials. The major excretion products were the hydrophilic product unknowns **2**, **3**, and **4**, which are probably water-soluble conjugates; these coeluted with extraneous compounds.

The method of reversed-phase HPLC for detection of carbaryl described here was similar to that of Strait et al. (1991) and Ward et al. (1987) in the use of UV detection and methanol/water as a mobile phase. However, by combining the HPLC method with the radioisotopic technique, we increased our detection sensitivity, thus

allowing more reliable identification and quantitation of the trace metabolites. This method simplified the sample extraction and cleanup process, decreased sample handling time, and reduced the risk of compound degradation. In addition, this method will positively identify and quantify the amount of the compounds even though coeluting with the extraneous material from the bark beetles. The method, therefore, has potential for a variety of applications for degradation and metabolism studies.

ACKNOWLEDGMENT

We thank Drs. N. Andrawes, W. C. Dauterman, J. F. Monahan, and R. Werner for a reviewing an earlier draft manuscript. We thank F. Sanchez for his assistance with HPLC techniques, Dr. N. Andrawes for samples of carbaryl and its metabolites, Dr. W. Dauterman for radiolabeled carbaryl, and Mrs. W. Hobbs for maintenance of the southern pine beetle colony. We thank C. S. Bradberry for technical assistance. This research was supported by USDA-FS, Pacific Northwest Research Station under Agreement PNW-86-526. This is Harbor Branch Oceanographic Institution Contribution No. 1005.

LITERATURE CITED

Andrawes, N. R.; Dorough, H. W. Metabolic fate of carbaryl-naphthyl-^{14}C in boll weevils and bollworms. *J. Econ Entomol.* 1967, *60*, 453–456.

Berisford, C. W.; Brady, U. E.; Fitzpatrick, G. E. Efficacy studies; prevention. In *Field and laboratory evaluations of insecticides for southern pine beetle control.* Hastings, F. L., Coster, J. E., Eds.; Forestry Service General Technical Report SE-21; U.S. Department of Agriculture: Washington, DC, 1981; pp 3–8.

Brayan, J. G.; Haddad, P. R.; Sharp, G, J.; Dilli, S. Determination of organophosphate pesticides and carbaryl on paddy rice by reversed-phase high-performance liquid chromatography. *J. Chromatogr.* 1988, *447*, 249–255.

Dorough, H. W.; Casida. J. E. Nature of certain carbamate metabolites of the insecticide Sevin. *J. Agric. Food Chem.* 1964, *12*, 294–304.

Hall, R. W.; Shea, P. J.; Haverty, M. J. Effectiveness of carbaryl and chlorpyrifos for protecting ponderosa pine trees from attack by the western pine beetle (Coleoptera: Scolytidae). *J. Econ. Entomol.* 1982, *75*, 504–508.

Jones, A. S.; Jones, L. A.; Hastings, F. L. A rapid, direct high-performance liquid chromatographic method for the simultaneous quantitative determination of carbaryl and α-naphthol in aqueous samples. *J. Agric. Food Chem.* 1982, *30*, 997–999.

Kawai, S.; Goto, K.; Kano, K; Kubota, T. Determination of carbaryl by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr.* 1988, *442*, 451–454.

Kuhr, R. J. Metabolism of carbamate insecticide chemicals in plants and insects. *J. Agric. Food Chem.* 1970, *18*, 1023–1030.

Page, M.; Haverty, M. I.; Richmond, C. E. *Residual activity of carbaryl protected lodgepole pine against mountain pine beetle, Dillon, Colorado, 1982–1983.* Forestry General Technical Report psw-375; U.S. Department of Agriculture: Washington, DC, 1985; 4 pp.

Schlagbauer, B. G. L.; Schlagbauer, A. W. J. Metabolism of carbamate pesticides, Literature analysis I. *Residue Rev.* 1972, *42*, 1–84.

Spittler, T. D.; Marafioti, R. A.; Helfman, G. W.; Morse, R. A. Determination of carbaryl in honeybees and pollen by high-performance liquid chromatography. *J. Chromatogr.* 1986, *352*, 439–443.

Strait, J. R.; Thornwall, G. C.; Ehrich, M. Sensitive high performance liquid chromatographic analysis for toxicological studies with carbaryl. *J. Agric. Food Chem.* 1991, *39*, 710–713.

Ward, S. A.; May, G.; Branch, R. A. Quantitative determination of carbaryl and carbaryl metabolites by reverse-phase high performance liquid chromatography. *J. Chromatogr.* 1987, *388*, 462–466.

Werner, R. A.; Hastings, F. L.; Holsten, E. H.; Jones, A. S. Carbaryl and lindane protect white spruce from attack by spruce beetles (Coleoptera; Scolytidae) for three growing seasons. *J. Econ. Entomol.* 1986, *79*, 1121–1124.

Received for review June 7, 1993. Accepted February 7, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1994.