Determination of Propoxur Residues in Whole Body Brown Tree Snakes

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Propoxur was extracted from brown tree snakes (*Boiga irregularis*) and analyzed by reversed-phase high-performance liquid chromatography. Propoxur was quantified by UV absorbance at 270 nm and fluorescence with excitation at 225 nm and emission at 305 nm. Recovery data were determined by analyzing propoxur-fortified blank homogenized tissue. The mean recovery of propoxur in whole body brown tree snakes was 86.7 ± 7.8% for the concentration range of 0.1 to 250 μg/g. The method limit of detection with fluorescence and UV detection was 9 and 100 ppb, respectively.

Keywords: *Propoxur (CAS No. 114-26-1, number supplied by author); brown tree snake; high-performance liquid chromatography; fluorescence detection; UV detection; tissue; matrix solid-phase dispersion*

INTRODUCTION

The brown tree snake (*Boiga irregularis*) is not indigenous to the island of Guam but was probably introduced accidentally after the end of World War II. Since then, this snake has been responsible for the extinction of nine species of native forest birds (Savidge, 1987) and the decline of several lizard species on Guam (Rodda and Fritts, 1992). The brown tree snake is mildly venomous and poses a health risk to children (Fritts et al., 1990), causes electrical power outages by climbing on wires (Fritts et al., 1987), and preys on domesticated birds (Fritts and McCoid, 1991). An integrated control strategy is being developed for these snakes, with chemical control being investigated as one possibility. Currently, propoxur is being investigated with rotenone and pyrethrins as a means for chemical control of brown tree snake populations on Guam. Each of these compounds is being tested for efficacy and, if utilized, would be used in a bait station targeted for the brown tree snake. Propoxur [(2-(1-methylethoxy)phenol methylcarbamate)] is a widely used cholinesterase inhibiting carbamate insecticide that is highly effective in controlling a variety of insect pests.

Rapid and reliable analytical methods are needed to determine propoxur, rotenone, and pyrethrin residues in brown tree snakes to help interpret the efficacy experiments and as a tool to potentially assess secondary hazards. Many methods exist for the determination of carbamates in various matrixes. For complex matrixes such as tissues, extensive sample cleanup and postcolumn derivitization are required to detect the analytes at concentrations below 50 ppb. A simple and efficient extraction technique was developed by scientists at the Louisiana State University to isolate drug and chemical residues from biological matrixes (Barker et al., 1989, 1993). The technique, described as matrix solid-phase dispersion (MSPD), consisted of thoroughly mixing a small portion of matrix with a sorbent (C-18, C-8, C-2, CN, silica, alumina, and Florisil) followed by extraction of the matrix/sorbent mixture with a small amount of solvent to extract the analyte of interest. MSPD has also been applied to the analysis of organochlorine pesticide and drug residues in shellfish, fish, bovine, swine, and chicken tissues as described in review articles by Barker et al. (1993) and Walker et al. (1993). MSPD has been used to determine oxamyl and methomyl in vegetation (Stafford and Lin, 1992), organochlorine and organophosphorus pesticides in vegetation (Torres et al., 1995, 1996), pyrethroids in vegetables (Ling and Huang, 1995), and polychlorinated biphenyls in fish (Ling et al., 1994). We applied this technique, combined with a simple solid-phase extraction (SPE) cleanup, to determine propoxur in whole body brown tree snakes. Fluorescence detection can be used to quantify propoxur at low levels without the use of postcolumn derivitization (García Sánchez et al., 1994). This work reports the development of a validated method for the assay of propoxur in brown tree snake tissue at concentrations of 0.1−250 μg/g.

MATERIALS AND METHODS

Samples. The samples consisted of whole brown tree snakes (control and treated) from the island of Guam. The control snakes were analyzed to ensure the absence of analyte or chromatographic interferences. The control snakes were fortified (see Fortification of Controls) with known amounts of propoxur for method development and quality control.

Apparatus. A Hewlett-Packard (Palo Alto, CA) 1090M high-performance liquid chromatography (HPLC) system equipped with a Hewlett-Packard computer workstation was operated at 40 °C with a Keystone ODS/H (Bellafonte, PA) analytical column (250 mm × 4.6 mm i.d.) and a guard column (15 mm × 4.6 mm i.d.). The HPLC was equipped with an

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ultraviolet/visible diode array detector used at 270 nm, and a SpectroVision Inc. FD-300 (Cambridge, MA) dual-monochromator fluorescence detector with an excitation wavelength of 225 nm and an emission wavelength of 305 nm was used to detect propoxur. The sample extracts and standard (75 μL) were chromatographed with a mobile phase consisting of a 1:1 mixture of acetonitrile and deionized water with a flow rate of 1.0 mL/min and a run time of 10 min. The solvents were filtered through a 0.45-μm nylon filter and degassed by sparging with helium.

Reagents. Liquid chromatography grade acetonitrile (Fischer Scientific, Denver, CO) and deionized water purified with a Milli-Q water purification system (Millipore, Bedford, MA) were combined at 7:3 (vol:vol) to prepare the extraction solution. Propoxur (99%) was obtained from Chem Service, Inc. (West Chester, PA). The extraction solution was used to reconstitute the final extracts and dilute calibration standards. Silica gel (32−63 mesh) and SPE C-18 columns (Isolute, 1 g with 6-mL reservoir) were obtained from Jones Chromatography (Lakewood, CO).

Concentrated standard solutions were prepared by accurately weighing 10.0 mg of propoxur into a 10-mL volumetric flask. The propoxur was dissolved in and diluted to volume with the extraction solution. The concentration of the standard was approximately 1 mg/mL (1000 μg/mL).

Calibration Curve. Calibration standard solutions for ultraviolet detection ranged from 1.0 to 250 μg/mL and for fluorescence detection ranged from 0.05 to 1.0 μg/mL. All calibration standard solutions were prepared in 10.0-mL volumetric flasks with the extraction solution as the diluent. For residue concentrations greater than 0.5 μg/g, UV detection was used. For residue concentrations less than 0.5 μg/g, fluorescence detection was used.

Extraction of Propoxur in Brown Tree Snakes. Whole brown tree snakes were cut into approximately 2-in. pieces. The pieces were frozen with liquid nitrogen in a stainless steel cylindrical container. The frozen snake was shattered into a powder with a steel piston. Once most of the tissue was powdered, the large unhomogenized pieces of tissue were removed. The powdered tissue was transferred to a separate container, and the remaining pieces were refrozen and shattered into powder and combined with the rest of the powdered sample (Sterner and Mauldin, 1995).

Screw top, graduated 15-mL centrifuge tubes were calibrated to 1.00 mL by adding extraction solution with a 1.00 mL-Hamilton syringe. The bottom of the meniscus was marked with a permanent marker.

A 2.0-g portion of powdered tissue was weighed into a small glass mortar. A 2.0-g portion of silica gel was added to the tissue. The tissue and silica gel were ground with a pestle until homogeneous. The silica gel/tissue mixture was carefully transferred into a 10-mL polypropylene syringe that contained a filter paper disk (1.5-cm diameter). The sorbent/tissue mixture was packed as tightly as possible by adding another filter paper disk to the top of the silica gel/tissue mixture and pushing with the plunger to form the column. The mortar and pestle were rinsed with approximately 5 mL of extraction solution which was loaded onto the top of the column of the silica gel/tissue mixture to extract the propoxur. Two additional 2-mL aliquots of extraction solution were then added. All of the eluant was collected in a 25-mL test tube, using the vacuum manifold at a flow of approximately 1−2 mL/min. A C-18 (1-g) solid-phase extraction column was conditioned with 4 mL of extraction solution. The eluant from the extraction step was eluted through the conditioned C-18 SPE column and was collected in a precalibrated centrifuge tube. Each 25-mL sample tube was rinsed two times with 2 mL of extraction solution and the rinsate loaded onto the respective SPE column. The solvent was evaporated from the final eluant by placing each centrifuge tube in a warm water bath and blowing a stream of nitrogen over the solution until the solution volume was less than 0.5 mL. The solution was diluted to the 1.00-mL calibration mark with extraction solution. Each sample was filtered through a 0.45-μm nylon filter before analyzing by HPLC. A separate filter and syringe were used for each sample.

Table 1. Fortification Levels and Percent Recoveries for Propoxur in Brown Tree Snake Tissue

	0.1 μg/g	1.0 μg/g	10 μg/g	100 μg/g	250 μg/g
replicates	6	5	6	5	5
mean	88.1	87.0	87.7	82.4	88.1
SD	10	4.7	9.9	3.9	9.0
CV	11	5.4	11	4.7	10

The operating conditions were adjusted to obtain optimum response and reproducibility. For 1:1 acetonitrile/water mobile phase, the retention time of propoxur was approximately 7.8 min. At the end of each analysis sequence, a column wash was performed by pumping acetonitrile through the guard column for approximately 20 min. With this procedure, the lifetime of the guard column was increased from $n = 30$ samples to $n = 80$ samples before it was necessary to change the guard column.

Fortification of Controls. Propoxur fortification standard solutions at 2500, 1000, 100, 10.0, and 1.00 μg/mL in acetone were used for fortifying the control tissue to evaluate analyte recovery. Each 2.00-g portion of control brown tree snake tissue was fortified at 250, 100, 10, 1.0, and 0.10 μg/g with 0.200-mL aliquots of the appropriate fortification standard solution. The acetone was allowed to evaporate for 20 min, and the extraction and cleanup procedure was followed as previously described.

RESULTS AND DISCUSSION

Instrumental Data. Two sets of calibration standard solutions were prepared ranging in concentrations from 1.0 to 250 μg/mL for UV detection and from 0.05 to 1.0 μg/mL for fluorescence detection. Each standard solution was injected twice, and a plot was constructed of propoxur chromatographic peak area response (y axis) versus propoxur concentrations (x axis). A linear regression was performed on each data set. For both UV and fluorescence detection, the correlation coefficients of peak response versus concentration were ≥0.9989. For UV detection, the log−log data demonstrated that peak response was proportional to concentration over the range of the calibration curves, indicating that single-point calibrations were valid from 1.0 to 250 μg/mL. For fluorescence detection, a linear relationship existed between propoxur chromatographic peak response and propoxur concentrations greater than 0.05 μg/mL; however, this relationship was not proportional. Single-point calibrations were not valid over this range of standard solution concentrations. Working standard solutions were used for fluorescence detection assays. Fluorescence detection was required when residues were less than the method limit of detection (MLOD) for UV detection. Typically, fluorescence was used when propoxur concentrations were less than 0.5 μg/g.

Instrument limit of detection (ILOD) was defined as the concentration of propoxur in the sample required to generate a signal equal to 3 times the baseline noise (measured peak-to-peak) observed in the standard sample. The ILOD was estimated from the mean chromatographic peak height of a propoxur standard solution (0.05 μg/mL for fluorescence and 1.0 μg/mL for UV) and the peak-to-peak noise observed from three reagent blank samples. The ILOD for UV and fluorescence detection was determined to be 0.12 and 0.014 μg/mL for propoxur.

Residue in Brown Tree Snakes. The recoveries of propoxur from fortified control brown tree snake

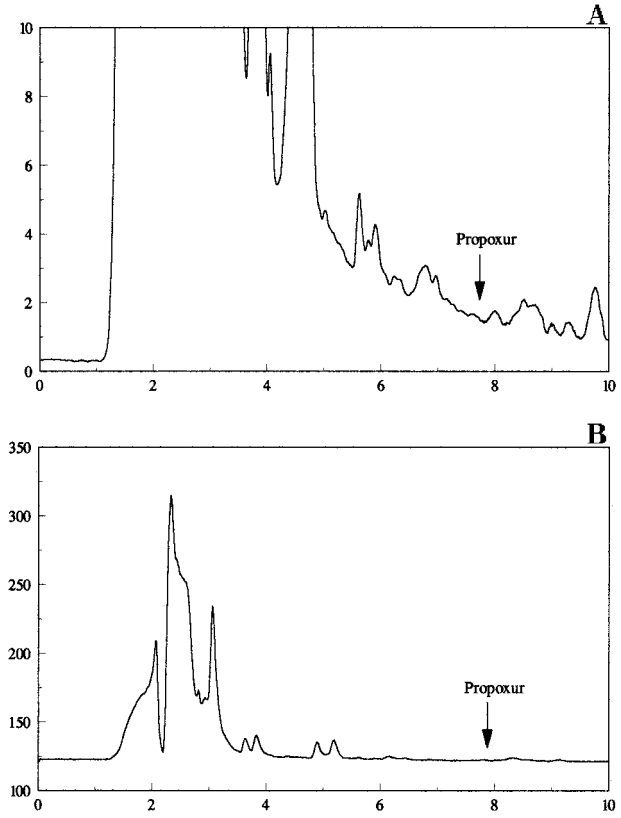

Figure 1. Chromatograms of a blank brown tree snake control sample extract with (A) UV detection and (B) fluorescence detection.

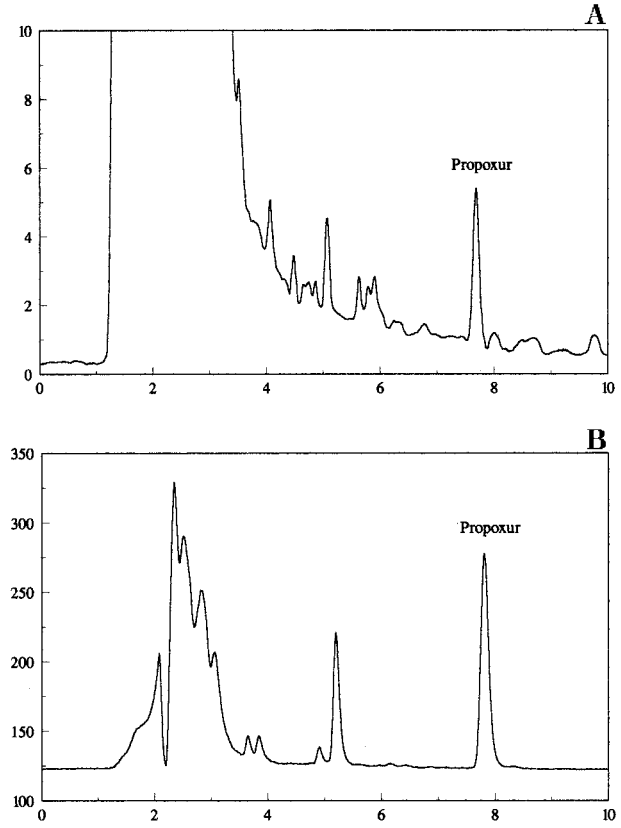

Figure 2. Chromatograms of a 1.0 μg/g propoxur-fortified control sample extracted and analyzed with (A) UV detection and (B) fluorescence detection.

samples obtained by the MSPD method are listed in Table 1. The mean recoveries ranged from 82.4 to 88.1%

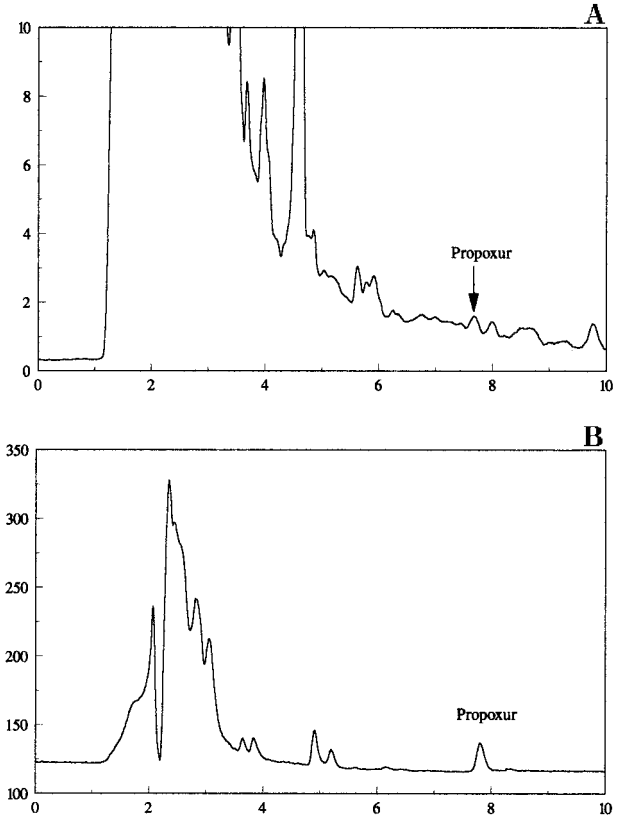

Figure 3. Chromatograms of a 0.10 μg/g propoxur-fortified control sample extracted and analyzed with (A) UV detection and (B) fluorescence detection.

Table 2. Incurred Residues from Brown Tree Snakes Exposed to Propoxur Formulations

sample	treatment	snake wt (g)	μg/g propoxur	mortality
	A. Orally Treated			
1	20 mg of Spam bait	124.0	134	died
2	20 mg of Spam bait	131.4	106	died
3	20 mg of Spam bait	136.8	116	survived
4	20 mg of Spam bait	91.0	141	died
5	0 mg of Spam bait	61.8	<MLOD	
6	0 mg of Spam bait	88.8	<MLOD	
	B. Dermally Treated			
7	80 mg/kg propoxur	45.9	21.4	died
8	80 mg/kg propoxur	71.1	19.4	died
9	80 mg/kg propoxur	85.9	0.770	survived
10	80 mg/kg propoxur	187.8	1.34	survived
11	80 mg/kg propoxur	66.2	0.713	survived
12	40 mg/kg propoxur	61.9	<MLOD	survived
13	40 mg/kg propoxur	266.8	19.2	died
14	40 mg/kg propoxur	36.6	17.8	died
15	40 mg/kg propoxur	94.0	15.3[a]	died
16	40 mg/kg propoxur	81.4	<MLOD	survived
17	0 mg/kg propoxur	101.4	<MLOD	
18	0 mg/kg propoxur	100.1	<MLOD	
19	0 mg/kg propoxur	146.6	<MLOD	

[a] Analyzed in triplicate.

with a CV of ≤11% for fortification levels of 0.10−250 μg/g. Chromatograms of a control sample extract (Figure 1) and extracts from control samples fortified at approximately 1.0 (Figure 2) and 0.10 μg/g (Figure 3) levels of propoxur are shown. From the attached chromatograms, no interferences were observed and fluorescence detection was more selective than UV and therefore provided a simpler chromatogram. Method limit of detection (MLOD) was defined as the concentration of propoxur in the sample required to generate a

signal equal to 3 times the baseline noise (measured peak-to-peak) observed in the control sample. The MLOD was estimated from the mean chromatographic peak height of propoxur in seven fortified samples (0.10 μg/g for fluorescence and 1.0 μg/g for UV) and the peak-to-peak noise observed from three control samples. The MLOD for UV detection was determined to be 0.10 μg/g for propoxur. The MLOD for fluorescence detection was determined to be 0.009 μg/g for propoxur.

The residue results of brown tree snakes from efficacy studies in which the snakes were either given propoxur-fortified baits orally or treated dermally with propoxur formulations are shown in Table 2. Samples were homogenized and assayed in duplicate. The method yielded very reproducible results over a wide range of incurred residues, as the mean coefficient of variation for all the samples listed in Table 1 was 6.0%. Incurred residues ranged from 134 μg/g to less than the MLOD. For dermal application, the snakes that survived contained propoxur residues that varied from <MLOD to 1.34 μg/g and the snakes that died contained propoxur residues from 15.3 to 21.4 μg/g. The quality control samples assayed with the orally and dermally treated snakes yielded mean recoveries of 85.4% ($n = 2$ at 100 μg/g) and 95.7% ($n = 12$ at 0.1 and 10 μg/g).

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