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Survey of Persistent Pesticide Residues in the Edible Tissues of Wild and Pond-raised Louisiana Crayfish and Their Habitat

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Procambarus clarkii and P. acutus acutus crayfish, the two most important species of an economically important crop in southern Louisiana, are commercially harvested from dual-cropped ponds (rice:crayfish) and from waters contiguous with the Atchafalaya River Basin ("Basin") and the Mississippi River. Approximately 135,000 acres in Louisiana are dedicated to crayfish culture with an annual harvest of about 60 million pounds valued at about $28 million (Anon, 1987). Catches of wild crayfish fluctuate greatly from one year to the next.

Crayfish are sold in-state, both alive for boiling and as peeled and deveined tail meat with attached hepatopancreatic tissue ("fat") for use in many "Cajun" dishes. Increased attempts to penetrate out-of-state and overseas markets have required confirmation of high quality products. Many countries, particularly Scandinavian, have considerably more stringent quality standards for microbial and chemical contamination than those in the U.S.

Procambarus spp. are known detritivores, which during their feeding may ingest xenobiotic contaminants adsorbed onto decaying vegetation and the sediment. Large quantities of persistent organochlorine pesticides had been applied to various Louisiana grain and fiber crops prior to their ban almost 20 years ago. Additionally, Louisiana may receive agricultural runoff from the fields in central U.S., since it is located at the outlet of the Mississippi River, which drains one-third of the continental U.S. Therefore, it is conceivable that crayfish may store persistent pesticides in their edible tissues, the abdominal muscle ("tail meat") and the hepatopancreas. Thus, this study investigated the quantity of persistent pesticides in the edible tissues, whether the source of the pesticides was the sediment or water in their environs, and whether the location or time of season harvested were important factors for residue concentrations.

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MATERIALS AND METHODS

Crayfish, sediment, and water were collected from several commercial ponds and the Basin at three times during the 1986 and 1987 production years, early, middle ("peak"), and late season.

Although weather, the ambition of the fishermen, and price dictated the season to a large extent, pond production seasons roughly correspond to "early" as late Dec. to late Feb., "peak" as Mar. to mid April, and "late" as late April to early June. Basin seasons generally run 2-4 weeks later.

Pond collection sites in 1986 were near Branch, Louisiana (NE Acadia Parish, AP), at the LSU Rice Experiment Station, Crowley, Louisiana (SE Acadia Parish, CRS), and near Breaux Bridge, Louisiana (NW St. Martin Parish, SMP), while wild samples were collected near Henderson, Louisiana (NE St. Martin Parish, UAB), and near Morgan City, Louisiana (SE St. Mary Parish, LAB). Pond sites in 1987 were located near Kaplan, Louisiana (N.Central Vermilion Parish, VP) and at the Crowley Rice Station, and the wild samples were collected at the same Basin sites.

Abdominal muscle and the hepatopancreas were removed from iced live crayfish and frozen separately at -20^{o}C prior to analysis. No attempt was made to separate the crayfish by sex, size, or species during the peeling procedure. White river crayfish (P. acutus acutus) were noticed only in the 1986 early and peak season in upper Basin and St. Martin Parish pond samples.

Three replicates of abdominal muscle and hepatopancreatic tissue for each year-time of season-location combination were extracted and purified prior to gas chromatographic analysis using U.S. FDA Pesticide Analytical Manual (PAM, Vol. 1, 211.14 a.d) fluorosil procedures modified by using 4% NaCl in the hepatopancreatic tissue samples instead of 2% in order to reduce emulsion formation. Concentrated final elutions were stored at 4^{o}C prior to analysis.

The AOAC (1984,14th ed., Section 29.001+) procedures for sediment clean-up were modified by adding the blended dried sediment, sodium sulfate, and 50:50 hexane: acetone solution to an Erlenmeyer flask, which was occasionally swirled to mix the materials.

Water samples were prepared using a modification of the U.S. FDA PAM (Section 10-A, revised 6/77) methylene chlorine extraction procedure. The water sample was shaken and filtered through petroleum ether-rinsed glass wool in order to remove dead algae and other suspended materials prior to extraction. Sediment and water samples were not replicated.

Solvent blanks and external standards were incorporated into each cycle preparation. Solvents of residue analysis grade, glass-distilled water, and reagent grade chemicals were used. Prepared samples were injected by a Precision Science (Baton Rouge, LA) GC 311V autosampler into a Tracor 540 Gas Chromatograph (Austin, TX) equipment with a Tracor electron capture detector (ECD) with a ^{63}Ni Source. Samples were eluted from DB-5 and DB-210 Megabore columns (J+W Scientific, Folsom, CA, 0.530 mm diameter x 15m length, 1.0 um film thickness) with ultra-high purity nitrogen (UHP N_2, 10 mL/min) to separate pesticides. Pesticides were detected by purging the ECD with an additional 40mL/min UHP nitrogen. The detector was maintained at 350°C and the injection port at 120°C. The GC was programmed to increase the oven temperature by 3°C/min after remaining at 120°C for 3 min until a final temperature of 210°C was attained. The column was held at 210°C for 5 min. Duplicate 0.3 microliter samples were injected and analyzed by a Spectra Physics 4100 (San Jose, CA) integrator.

RESULTS AND DISCUSSION

Crayfish abdominal muscle and hepatopancreas, sediment and water collected from four dual-cropped ponds and two Atchafalaya River Basin locations during the 1986 and 1987 production years were analyzed for persistent and organochlorine pesticides. Pesticide residue values obtained from the more sensitive column and confirmed are presented in Table 1. External standards were used for identification and quantification.

The only pesticide residues detected in occasional tissue samples were DDD and DDE. DDD residue levels in the abdominal muscle were detected only in the early 1987 VP ponds, the peak 1987 LAB, and UAB samples. DDE was confirmed in the abdominal muscle only from the early 1987 VP samples and in the hepatopancreas from the early 1986 LAB and early 1987 VP samples. Neither DDD nor DDE were detected in water or sediment samples. No confirmable quantities of dieldrin, endrin, heptachlor, heptachlor epoxide, or Mirex^R were detected in any tissue or environmental sample.

The relatively high readings (0.311 mg/kg p,p' DDD detected in the early 1987 abdominal muscle VP samples, 0.364 p,p'DDD in the peak 1987 UAB hepatopancreas, and 0.109 in the late 1987 VP hepatopancreas) were not confirmed on the second column and most likely represent co-eluting artifacts.

Markin et al. (1972) noted no significant difference between reported levels of previous pesticide usage and the commercial catch of P. clarkii from a variety of locations in southern Louisiana. The crayfish accumulated up to 0.07 mg/kg (whole body) of Mirex^R, an extensively used fire ant pesticide, and between 0.02 - 0.44 mg/kg DDT. Orconectes lancifer from two NE Louisiana lakes -L. Providence (near an urban area) and L.

Bruin (surrounded by farm land)- accumulated 0.13 and 0.03 mg/kg DDE, respectively. No other targeted organochlorine pesticides were detected (Niethammer et al., 1984). P. clarkii samples from three areas in southern Louisiana with histories of moderate persistent pesticide applications contained only trace amounts of chlordane in a 1978 sample and no detectable amounts of the 15 targeted organochlorine pesticides during the two years studied (Dowd et al., 1985).

Table 1. Pesticide residues in crayfish, Procambarus clarkii abdominal muscle and hepatopancreatic tissue.

Tissue	Year	Sample[1]	DB-5[2]	DB-210[2]
			DDD	
Abdominal	1986	all	none detected	none detected
Muscle	1987	LAB P (1)	0.040 p,p' 0.003 o,p'	≤0.002 o,p' p,p'
		UAB P (1)	0.040 o,p'	≤0.002 o,p'
		VP E (2)	0.311 p,p' 0.001 o,p'	≤0.002 o,p' p,p'
		all others	none detected	none detected
Hepato-	1986	LAB P (1)	0.009 o,p'	≤0.002 o,p'
pancreas		UAB L (1)	0.003 p,p'	≤0.002 p,p'
	1987	UAB P (1)	0.364 p,p'	≤0.002 p,p'
		VP L (1)	0.109 p,p'	≤0.002 p,p'
		all others	none detected	none detected
			DDE	
Abdominal	1986	all	none detected	none detected
Muscle	1987	VP E (1)	0.011 p, p'	≤0.002 p,p'
		all others	none detected	none detected
Hepato-	1986	LAB E (1)	0.018 p,p'	≤0.002 p,p'
pancreas		all others	none detected	none detected
	1987	VP E (1)	0.043 p,p' 0.019 o,p'	≤0.002 p,p' o,p'

[1]Sample:the first 2 or 3 letters indicate the location LAB (Lower Atchafalaya River Basin), UAB (Upper Atchafalaya River Basin), VP (Vermilion Parish pond); the next letter indicates the time of season harvested E (early), P (peak), L (late); the number indicates the number of samples with detectable confirmed residues of the triplicate samples.

[2]DB-5 and DB-210 indicate the Megabore columns that were used for separation of the pesticide residues. The concentrations are in mg/kg using external standards with an electron capture detector.

Since crayfish are detritivores, the presence of pesticides in the sediment may be a source of residues in the tissues. Detritus from several locations in the Apalachicola River, which is very similar to the Atchafalaya River in Louisiana, adsorbed up to 0.078 mg/kg DDT, 0.04 DDE, 0.012 DDD, 0.0017 dieldrin, and 0.0017 heptachlor epoxide, while up to 0.025 mg/kg DDD, 0.0035 DDE, and 0.0009 dieldrin were adsorbed to five-grain (≤250 mm) particles (Elder and Mattraw, Jr. 1984).

Residue concentrations in the sediment of DDT, DDD, DDE, dieldrin, endrin, heptachlor epoxide, and Mirex^{R} were reported to be less than 1 ug/kg in three NE Louisiana lakes sediments (Niethammer et al, 1984). Herring & Cotton (1970) reported

nearly 10 fold more DDT, 200-fold more DDT, and similar amounts of DDE in sediments from Mississippi lakes (outside the levee), which drained farm land, compared with oxbow labs inside the levee.

Sediment samples from several southern Louisiana locations, near several of the collection sites in the present study, generally contained less than 0.001 mg/kg of the common organochlorine pesticides and their degradation products (U.S. Dept. Interior et al 1980, 1982, U.S. Geolog. Survey 1981). Organochlorine pesticides have low water solubilities and, therefore, are seldom present in water samples (Edwards, 1973), which was confirmed in this study.

Persistent organochlorine pesticide residue concentrations in the edible tissue of the commercially important Louisiana crayfish were very low. Simultaneous with the ban of a majority of the persistent pesticides in the mid-1970s was the revocation of the recommended tolerances (for DDT and related compounds was 7.0 mg/kg in meat, poultry and fish (WHO/FAO, 1973)). Although the half-life for these compounds is long, the number of average 95% disappearance cycles that may have occurred since their ban (1.5 <for DDT> to 5 <for aldrin>, Edwards 1973), supplemented by fungal and bacterial activity in soils and sediments at the warm year-round temperatures of Louisiana, contributed to the low concentration of residues found.

The small amount of persistent pesticides that may have entered the crayfish from the ingestion of food and sediment or through the gills would be readily detoxified by the mixed function oxidase system found in the hepatopancreatic tissue, green gland, and other organs (Matsumura, 1985). From the aspect of human consumption, Louisiana crayfish appear to be relatively free of anthropogenetic persistent pesticide residues.

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