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Detection and Quantification of Insecticides in Shrimp Grown in a Coastal Farm in Sonora, Mexico

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Over many years a great number of pesticides have been used to control a variety of pests that affect agriculture. There are many routes by which pesticides may reach the human diet. Drinking water can be contaminated as a result of runoff in agricultural fields or industrial wastes. Soil in which different kinds of vegetables are grown, may acquire pesticide residues from contaminated water or by direct application of these agrochemicals. Coastal lagoons might get exposed to pesticide residues too. The ecosystems of major shrimp production in México are located along the subtropical Pacific Ocean. However, these have been affected due to increased anthropogenic wastes, such as municipal and agricultural sewage, that contain organochlorine and organophosphorous insecticides (Botello et al. 2000).

In Mexico, a number of studies have been performed on pesticide contamination of coastal lagoons and shrimp farms. Estuarine Urías in Mazatlán, Sinaloa (Galindo et al. 1987), coastal lagoon Huizache Caimanero in Sonora (Galindo et al. 1997), and coastal lagoon Carretas-Pereira, Chiapas (Botello et al. 2000) are some of the sites along the Mexican Pacific coast in which a variety of pesticides have been found and associated with high mortality during shrimp farming, a 600 million dollar/year industry for Mexico. Therefore, the objectives of this study were to quantify residues of organochlorine and organophosphorous insecticides in water and sediment from the estuary La Atanasia-Santo Domingo in Cajeme, Sonora, Mexico. In addition, pesticide levels were determined in the water, sediment and shrimp from a shrimp farming park adjacent to this zone.

MATERIALS AND METHODS

The present study was carried out on cultured shrimp obtained from a shrimp farming park adjacent to the estuary La Atanasia-Santo Domingo located (27°10' N latitude, 110°10' W longitude) in Cajeme, Sonora, México.

Shrimp samples (1 kg each) were collected from 3 ponds selected from every shrimp farm included in this study, from September to December 2000. Shrimp tails were separated from heads and stored at –20°C for further analysis. Extracts from shrimp samples were obtained according to Galindo et al. (1999) with some modifications.

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The extract was purified using a 350 mm x 20 mm silica gel / aluminum oxide / florisil / sodium sulfate anhydrous (4 + 4 + 2 + 4) column. Purified extracts were concentrated to 2 mL in a rotoevaporator and analyzed by GC, according to the procedures recommended by the APHA-AWWA-WPCF (1998), using a Varian 3800 model GC equipped with a 30m x 0.32 mm Durabon 608 column and an electron capture detector (ECD). Identification of compounds was deduced from their retention times, and quantification was based on comparisons of peak area with responses from reference standards. Reference standards used were lindane, 4'-4-DDD, 4'-4-DDE, methoxychlor, endrin, 4'-4-DDT, heptachlor, endosulfan, malathion, parathion, and chlorpyrifos. Recovery yields obtained in the present study were 99.6 ± 9.3 % and the detection limit was 1x 10^{-12}g.

In order to determine the mutagenic potential of insecticide-containing shrimp, 10 g of ground dried shrimp and 50 mL hexane-acetone (1:1 v/v) were combined and agitated with a wrist action shaker during 1 h. The mesh was filtered and the filtrate was evaporated to dryness in the presence of nitrogen. Extracts were re-suspended and serially diluted with DMSO and tested for mutagenicity.

Mutagenic potential was assayed using the standard plate incorporation procedure described by Maron and Ames (1983), using *Salmonella* tester strains TA98 and TA100, with and without metabolic activation (S9). The S9 mix (Aroclor 1254-induced, Sprague-Dawley male rat liver in 0.154 M KCl solution) used was purchased from Molecular Toxicology, Inc. (Annapolis, MD, USA). Briefly, 100 µl of sample was combined with 100 µl of bacterial culture and 500 µl of S9 mix (containing 4% S9 preparation) into a test tube containing 2.0 ml of top agar. This mixture was poured onto minimal glucose agar plates and incubated at 37°C for 48 hours. The number of revertants was counted using a Bactrovic colony counter, model C-110, New Brunswick Scientific Co., New Brunswick, N.J., and compared against the controls. The controls were different AFB_1 concentrations for both tester strains. Bacteria, that was kindly provided by Dr. Bruce Ames, University of California, Berkeley, CA, were checked for alterations and spontaneous reversions following the procedures of Maron and Ames (1983). All the assays were carried out in triplicate.

RESULTS AND DISCUSSION

Results of this research are summarized in Figures 1-2 and Table 1.

All insecticides sought (lindane, 4'-4-DDD, 4'-4-DDE, methoxychlor, endrin, 4'-4-DDT, heptachlor, endosulfan, malathion, parathion, and chlorpyrifos) were detected (Figures 1-2). Regardless of both the source and month, the insecticides found at higher concentrations in shrimp were malathion and parathion (up to 35 and 12 ng/g, respectively). It is important to note that insecticides detected in shrimp, such as lindane and DDT and its metabolites are restricted in Mexico. This restriction is based, according to the Instituto Nacional de Ecología (National Institute of Ecology), on their high risk for human health, high persistence and bioaccumulation properties. These insecticides should be used only in sanitary campaigns.

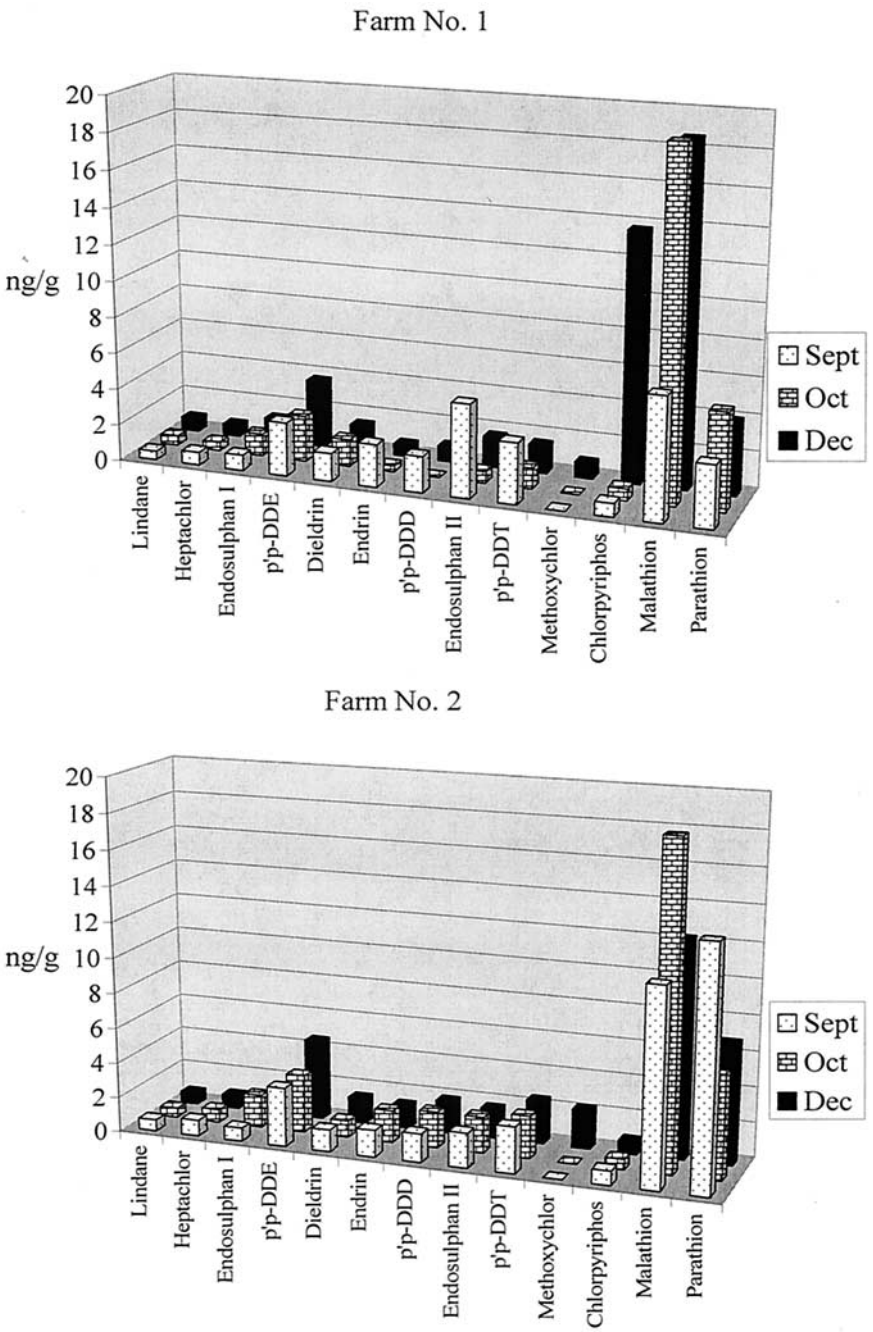

Figure 1. Insecticides levels found in shrimp cultivated in farms 1 and 2 from a shrimp farming park at Sonora, Mexico.

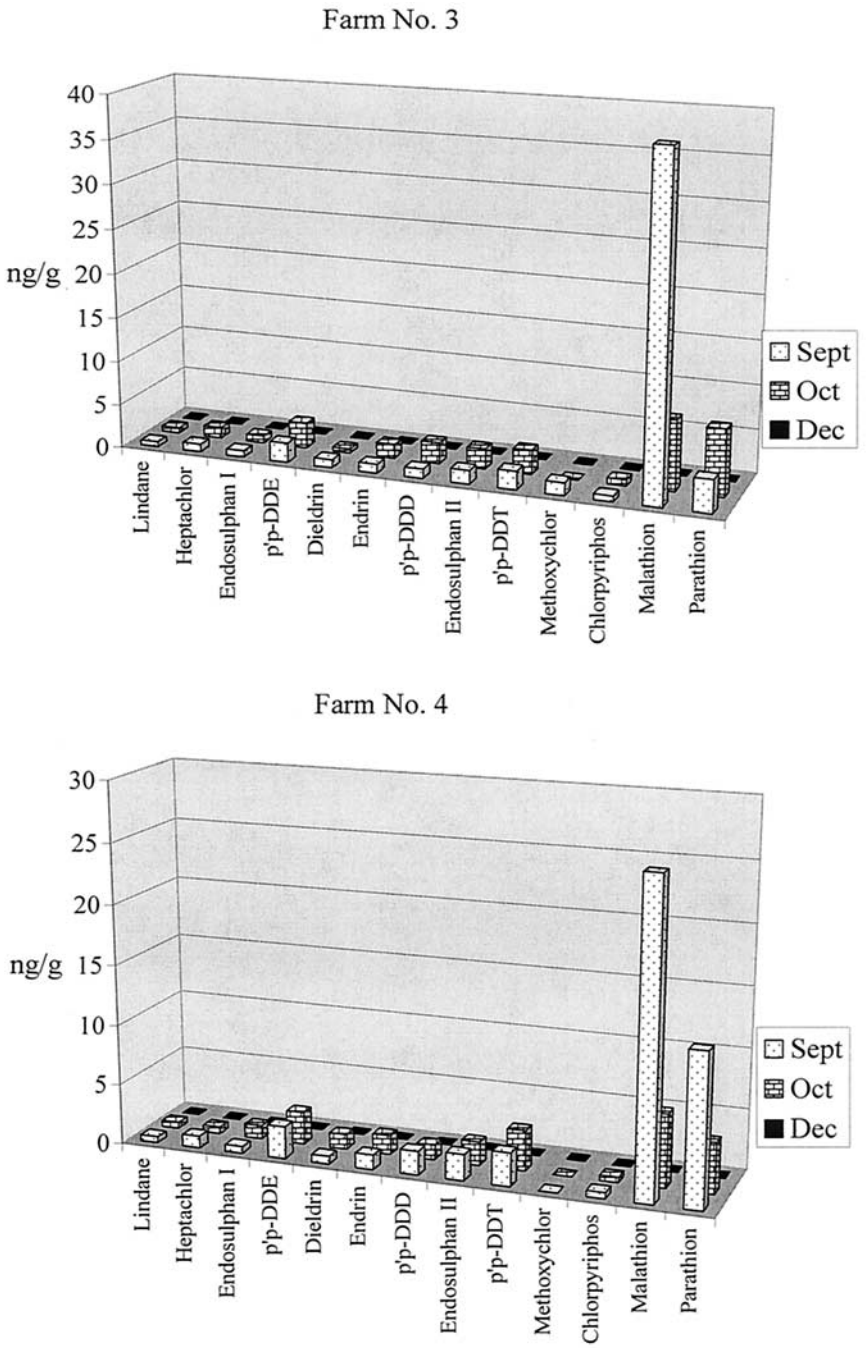

Figure 2. Insecticides levels found in shrimp cultivated in farms 3 and 4 from a shrimp farming park at Sonora, Mexico

Chlorpyrifos was found in significant concentrations (> 13 ng/g) in shrimp from farm 1, but only in December. Due to its fast biodegradation, these findings suggest recent use of this agent in adjacent agricultural fields. Other insecticides found at lower levels (< 4 ng/g) were endrin, dieldrin and DDT metabolites.

Table 1. Mutagenic potential of insecticide-contaminated farmed shrimp (average[a] TA100 and TA98 revertants / plate, with S9).

	Extract dilution			
Shrimp source	1×10^{0}	1×10^{-1}	1×10^{-2}	1×10^{-3}
TA98 w/S9				
Farm 1	20 ± 3	21 ± 4	20 ± 4	28 ± 2
Farm 2	17 ± 1	23 ± 3	21± 1	29 ± 3
Farm 3	25 ± 4	22 ± 2	25 ± 2	28 ± 4
Farm 4	21 ± 4	19 ± 2	19 ± 5	28 ± 4
TA98 w/o S9				
Farm 1	18 ± 1	17 ± 2	16 ± 0	25 ± 0
Farm 2	20 ± 3	16 ± 3	15 ± 1	25 ± 2
Farm 3	22 ± 3	18 ± 2	16 ± 1	24 ± 2
Farm 4	29 ± 2	21 ± 4	21 ± 3	20 ± 3
TA100 w/S9				
Farm 1	95 ± 6	120 ± 7	87 ± 32	123 ± 22
Farm 2	123 ± 13	118 ± 20	106 ± 1	105 ± 17
Farm 3	120 ± 18	83 ± 9	87 ± 15	100 ± 24
Farm 4	101 ± 13	105 ± 31	103 ± 14	117 ± 12
TA100 w/o S9				
Farm 1	140 ± 11	117 ± 15	126 ± 3	160 ± 17
Farm 2	122 ± 22	128 ± 10	121 ± 11	109 ± 11
Farm 3	103 ± 10	112 ± 5	112 ± 6	114 ± 16
Farm 4	122 ± 19	97 ± 15	96 ± 11	108 ± 15

AFB_1 (used as positive control) tested at 10, 100, and 1000 ng/plate induced (TA98) 33 ± 8, 75 ± 9, 825 ± 35, and (TA100) 153 ± 9, 251 ± 26, 1791 ± 62, revertants/plate. Spontaneous revertants were 25 ± 7 and 125 ± 19 for TA98 and TA100, respectively.

[a] Values are means of three replicates ± SD.

Insecticide levels for shrimp from farms 3 and 4 during December were not obtained due to emergency harvesting events. Even though we could detect all insecticides sought, the levels found were still below the FDA action levels: 300 ng/g for dieldrin, 300 ng/g for chlordane, 5000 ng/g for DDT and its metabolites, and 300 ng/g for heptachlor (FDA and EPA 1998). However, these levels have been reported to cause toxic effects in shrimp, such us lack of osmoregulation, decreased glycogen synthesis, increased respiration rate (Galindo et al. 1996; Galindo et al. 2000), and reduction of cholinesterase activity (Shannon et al. 1999; Diaz 2000). The latter damages the nervous system and may cause death. Therefore, the presence of toxic levels of insecticides, along with other factors (viral and bacterial diseases, climatic conditions) might have contributed to low farmed-shrimp yields achieved

during the last three farming seasons (personal communications from shrimp farmers).

To look for evidence of pesticide-contaminated shrimp being a potential source of health risk for consumer, the mutagenic potential of farmed shrimp tissue was determined. Even though none of the pesticide-contaminated shrimp samples were mutagenic in this assay (Table 1), a larger and more varied number of toxicological studies should be carried out in order to appropriately assess the potential risk for consumers.

Based on the results from this study, shrimp that are grown in south Sonora, Mexico contained pesticide residues in levels that might contribute to reduction in shrimp production and may represent a risk for consumer health, pending additional toxicity testing.

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REFERENCES

APHA-AWWA-WPCF (1998) Standard methods for the examination of water and wastewater. 20th edition, Chapter 4, Madrid Spain, p 6-171

Botello AV, Rueda-Quintana L, Diaz-Gonzalez G, Toledo A (2000) Persistent organochlorine pesticides (POPs) in coastal lagoons of the subtropical Mexican Pacific. Bull Environ Contam Toxicol 64: 390-397

Diaz A (2000) Biochemical responses in Penaeids cuased by contaminants. Aquaculture 191:163-168

FDA and EPA (1998) FDA & EPA Guidance levels. Food & Drug Administration. Center for Food Safety & Nutrition. Fish and Fisheries Products. Hazards and Control Guide. Appendix 5

Galindo G, Dalla L, Lazcano G, Rivas H (2000) Enzimatic and osmoregulative alterations in white shrimp *Litopenaeus vannamei* exposed to pesticides. Chemosphere 5:233-237

Galindo G, Fossato VU, Villagrana C, Dolci F (1999) Pesticides in water, sediments and shrimp from a coastal lagoon off the Gulf of California. Mar Pollut Bull 5:838-841

Galindo G, Medina A, Villagrana C (1996) Toxic effects of organochlorine pesticides on *Penaeus vannamei* shrimps in Sinaloa, Mexico. Chemosphere 5:567-575

Galindo G, Medina A, Villagrana C, Ibarra L (1997) Environmental and pollution condition of Huizache-Caimanero lagoon, in the north-west of Mexico. Mar Pollut Bull 5:1072-1077

Galindo G, Mexia J, Heredia L (1987) Contaminación en el camarón. Niveles y tipos de contaminación por plaguicidas en el camarón (*Penaeus sp.*) y en el agua del estero Urías, Mazatlán, Sinaloa. Boletín Ciencias del Mar 9:32-36

Instituto Nacional de Ecología (1998) Lista de plaguicidas restringidos en México. Diario Oficial de la Federación, Tomo DLXXV No.2, México, DF, p 524

Maron M, Ames BN (1983) Revised methods for the Salmonella mutagenicity test. Mutat Res 113:173-215

Shannon A, Fulton M, Key P (1999) The sensitivity of grass shrimp, *Palaemonetes pugio*, embryos to organophosphate pesticide induced acetylcholinesterase inhibition. Aquat Toxicol 48:127-134