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Insecticide Residues in Stored Grains in Sonora, Mexico: Quantification and Toxicity Testing

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Abstract Food safety has acquired great attention by food importer and exporters. Food rejection or acceptance across international borders is based on the compliance with international food regulations. Due to the lack of recent data on pesticide residues in Mexican grains, this study focused on detecting and quantifying insecticide residues in stored wheat, corn, chickpeas, and beans, as well as to determine their mutagenic potential. Grains were sampled from primary storage sites in Sonora, Mexico. Malathion, chlorpyrifos, deltamethrin, cypermethrin, 4,4-DDE, 4,4-DDD and 4,4-DDT were analyzed in 135 samples. Grain samples were not mutagenic and most pesticide levels were within regulation limits.

Keywords Insecticides · Grains · Toxicity testing · Ames test

In maintaining the quality of stored grains, one of the most important and prevailing problems is the great diversity of insects adapted to such an environment (Marei et al. 1995). People are commonly exposed to insecticides throughout pest control treatment of homes, lawns, gardens, workplaces, and commercial agricultural fields (Wagner et al. 2003). Insecticide application means that residues may accumulate in food and exceed the tolerance limit (Papadopoulou and Tomazou 1991).

Experimental studies of chronic exposure to insecticides have revealed an increased risk for neurotoxic, carcinogenic, teratogenic, and mutagenic effects, among others (Kamrin 1997). Most of the findings on the mutagenic effects of malathion are supported by in vitro studies (Galloway et al. 1987; Herath et al. 1989; Blasiak et al. 1999). Although the state of Sonora is considered one of the main producers of grains in Mexico, there are no official reports of pesticide usage in cultivated or stored grains. This information is crucial for grain producers marketing in the United States of America. Further, insecticide residues in Mexican grains are a major issue for importers.

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Materials and Methods

In order to obtain information about grain maintenance, hygiene practices, and type of insecticides used, a survey was designed and applied to the person responsible of every grain storage facility included in this study. Specifically, the survey was used to gather information on the insecticides used to prepare the storage facility prior to grain reception, those used during grain storage, as well as those used on the outdoor facilities. Information on pesticide application frequency and techniques used, as well as, on the personal protective equipment used by insecticide appliers were searched.

Wheat (n = 119), corn (n = 4), chickpea (n = 10), and bean (n = 2) samples were obtained from three geographical zones in the state of Sonora, Mexico. In the north: San Luis Rio Colorado and Caborca (three facilities); in the central region: Hermosillo (six facilities); and in the south: Vicam, Cajeme, Navojoa and Huatabampo (twelve facilities). In order to obtain information from different periods or insecticide application practices, samples were collected during three seasons of the year (May–June 2000, September–October 2000, and February–March 2001).

Sampling of grains was performed using a deep bin probe. The probe was introduced in different places of the storage bin to obtain a 4 kg representative and homogenous sample. The 4 kg sample was placed in a plastic bag and labeled. Samples were transported to the laboratory and divided using a Boerner divider to reduce sample weight to 1 kg. Grain samples were cleaned using a 3.18 mm or 8/64″ triangular sieve. Light material such as seeds and peel was separated from clean grain using a grain blower (No. 757 South, Dakota USA). Samples were handled individually and stored at −20°C until further analysis. Grain samples were grounded using a Laboratory Mill 3100 and sieved through a No. 60 mesh to homogenize particle size prior to extraction of insecticides.

The extraction of insecticide residues from grains was carried out according to Joia et al. (1985). 4,4-DDE, 4,4-DDD and 4,4-DDT (Chem Service, West Chester PA.), deltamethrin and cypermethrin (Riedel-de Haën GmbH, Seelze), as well as malathion and chlorpyrifos (Chem Service, West Chester PA.) were used as reference standards for trichlorodifenylethane metabolites, pyrethroids, and organophosphorus insecticides, respectively. All chemicals used were HPLC or chromatographic grade.

Detection and quantification of insecticide residues in grain extracts was performed by gas chromatography using a Varian CP-3800 GC model equipped with a DB-5 semi-polar, 30 mm × 0.32 mm column (J&W Scientific CA. USA) and an electron capture detector (ECD). Ultra high purity (99.999%) helium was used as a carrier with a flow rate of 30 mL/min, output gas pressure was 80 psi, injection and detection temperatures were 250°C and 300°C, respectively. Recovery yields obtained in the present study were 84.22%, 81.37%, and 74.58% for pyrethroids, organochlorides, and organophosphorus insecticides, respectively. Detection limit for 4, 4-DDT and metabolites was 1×10^{-12} g.

In order to determine the mutagenic potential of stored corn, wheat, chickpeas, and beans, in Sonora, Mexico, 50 g of ground grain 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 strain TA98 with and without metabolic activation (S9). The S9 mix (Aroclor 1254-induced, Sprague-Dawley male rat liver in 0.154 M KCl solution) was purchased from Molecular Toxicology, Inc. (Annapolis, MD, USA). Briefly, 100 µl of sample were 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 h. The number of revertants was obtained using a Bactrovic colony counter, model C-110, New Brunswick Scientific Co., New Brunswick, NJ, and compared against the controls. The controls were different AFB_1 concentrations. Bacteria, 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 from the survey indicated that in both, northern and southern Sonora, malathion was the insecticide most used in storage facilities and on outdoor facilities. Aluminum phosphide was the insecticide used for the maintenance of the grain. On the other hand, in the central zone of Sonora, malathion, deltamethrin, and aluminum phosphide were used inside the grain storage facility; for outdoor facilities cypermethrin was used.

The survey found that workers that apply insecticides in grain storages do not use the appropriate protective equipment. In addition, 2% of the grain stored used a larger dose than recommended, which might provide a potential for harmful effects on the consumer. The methods for insecticide application most widely used were spraying and thermonebulization.

Insecticides found in this study were malathion, chlorpyrifos, deltamethrin, cypermethrin, as well as DDT metabolites (4,4-DDE, 4,4-DDD and 4,4-DDT); presence of the latter might be due to residuality in the fields. Of the 135 samples collected, 60% were from the south, 32% from the north, and 8% from the central zone. The levels of the different insecticides residues found in grain flour are shown in Table 1. All insecticide residues were below the maximum limit permitted for stored grains, with the exception of cypermethrin (CICOPLAFEST 1998; FAO/WHO 1999).

In order to evaluate the genotoxic potential of the insecticide-containing grain, extracts from whole grains were obtained and tested for their mutagenic potential against *Salmonella* tester strain TA98 using the Ames test. The mutagenic potential of extracts obtained from the four

Table 1 Concentration of insecticide residues[1] found in whole wheat, corn, beans, and chickpeas grains, stored in the state of Sonora, Mexico

Insecticide	Maize (ng/g) n = 4	Beans (ng/g) n = 2	Chickpeas (ng/g) n = 10	Wheat (ng/g) n = 119
Malathion	2.2 ± 0.8	25.3 ± 15.4	22.6 ± 19.7	11.1 ± 11.3
Chlorpyrifos	7.9 ± 5.7	ND	1.0 ± 0.4	5.8 ± 5.8
Deltamethrin	76.6 ± 14.8	114.1 ± 38.2	113.4 ± 55	97.7 ± 179
Cypermethrin	ND	ND	365.7 ± 252.9	70.9 ± 126.1
4,4-DDT	ND	ND	2.0 ± 0.7	4.0 ± 2.1
4,4-DDD	ND	ND	ND	2.2 ± 1.6
4,4-DDE	ND	ND	ND	11.9 ± 31

[1] Values are means of three replicates ± SD

ND = Not Detected

Table 2 Mutagenic potential of pesticide-contaminated grains harvested and stored in Sonora, Mexico (average[a] TA100 and TA98 revertants/plate, with S9)

Grain	Extract dilution		
	1×10^{0}	1×10^{-1}	1×10^{-2}
TA98 w/S9			
Beans	52 ± 3	51 ± 6	45 ± 1
Corn	59 ± 12	66 ± 2	61 ± 12
Wheat	63 ± 4	67 ± 11	66 ± 3
Chickpeas	68 ± 11	75 ± 7	66 ± 9
TA98 w/o S9			
Beans	51 ± 7	63 ± 9	64 ± 4
Corn	53 ± 4	65 ± 10	–
Wheat	101 ± 16	82 ± 4	112 ± 8
Chickpeas	51 ± 6	67 ± 23	79 ± 3

AFB_1 (used as positive control) tested at 50, 500, and 1,000 ng/plate induced 171 ± 28, 417 ± 56, 1,187 ± 169, and 51 ± 3, 55 ± 14, 45 ± 5 revertants/plate in the presence and absence of metabolic activation (S9), respectively. Spontaneous revertants with and without S9 were 46 ± 4 and 47 ± 5, respectively

[a] Values are means of three replicates ± SD

Table 3 Mutagenic potential of pure insecticides that were found as contaminants residues in whole grains stored in the state of Sonora, Mexico

Insecticide	TA98 w/S9	TA98 w/o S9
Malathion (ng/plate)		
12.5	36 ± 5	74 ± 7
25	38 ± 5	81 ± 7
50	53 ± 6	110 ± 7
100	74 ± 8	129 ± 7
Chlorpyrifos (ng/plate)		
1.25	21 ± 6	68 ± 11
2.5	22 ± 3	87 ± 13
5	29 ± 3	108 ± 5
10	31 ± 8	85 ± 6
4,4-DDD (ng/plate)		
1.25	14 ± 3	17 ± 3
2.5	10 ± 3	22 ± 4
5	17 ± 3	39 ± 3
10	28 ± 3	52 ± 7

1-NP (used as positive control) tested at 10 μl induced 422 ± 28 revertants/plate. Spontaneous revertants with and without S9 were 46 ± 4 and 47 ± 5, respectively

[a] Values are means of three replicates ± SD

different grains was determined in the presence and absence of metabolic activation (S9) (Table 2). The inclusion of the S9 mix in the assay followed the purpose of detecting those genotoxic compounds in the tested grains that require bioactivation in order to be mutagenic. As shown in Table 2, none of the extracts were mutagenic to tested bacterial strain.

In order to detect genotoxic compounds present in tested grains, which might be deactivated by the S9 mix and loose their mutagenicity, extracts from the grains were assayed in the absence of metabolic activation. Similar results were observed when grain extracts were assayed in the absence of S9 mix (Table 2).

Due to failure in detecting mutagenicity in the assayed extracts from insecticide-containing grains, further mutagencity testing was performed on malathion, chlorpyrifos (both as organophosphorus representatives), and 4,4-DDD (as organochlorine representative) contaminated grains but in their pure form and at concentrations found as contaminants. Following Kado et al. (1983), for this assay a pre-incubation of S9 mix, tester strain culture, and the tested insecticide was done prior to top agar. As shown in Table 3, the three insecticides tested were mutagenic in the absence of metabolic activation (S9), however, when they were in contact with metabolic enzymes only malathion resulted mutagenic against tester strain. Since none of the tested insecticide-grain extracts were mutagenic and based on data obtained from testing the mutagenic potential of pure insecticides, these results suggest that compounds that interfere with the mutagenicity of insecticides might be present in tested grains. Previous research studies have shown evidence of the existence of antimutagenic compounds in maize. Burgos-Hernández et al. (2001a, b; 2002), described the presence of compounds in corn that interfered with the mutagenicity of aflatoxin B_1.

Finally, the evidence of the possible presence of insecticide-mutagenicity interfering compounds in grains and results from our study regarding the low concentration of pesticides found in stored grain might suggest that the grain stored in Sonora, Mexico does not pose a risk for genotoxicity for the consumer; however, further toxicological studies must be performed for a full risk assessment.

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