Analysis before and after Cooking Processes of a Trace Chlorpyrifos Spiked in Polished Rice

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Polished rice grains were fortified with 500 ppb of chlorpyrifos. The fortified residues were analyzed by gas chromatography with a nitrogen–phosphorus detector or a Hall detector. Chlorpyrifos and its breakdown product 3,5,6-trichloro-2-pyridinol were recovered from fortified rice grains in the levels of 456 and 3.4 ppb, respectively. Washing rice grains with water removed approximately 60% of the chlorpyrifos residues. Fortified chlorpyrifos was found in cooked rice at a level of 130 ppb, which was approximately 30% of the original quantity. Acid treatment was required to remove chlorpyrifos bound to proteins or carbohydrates in the cooked rice.

Heavy use of pesticides on farm fields has begun to receive much attention because pesticide residues in food commodities may be hazardous to human health. Therefore, many researchers have studied how to remove pesticide residues from food products (Liska and Stadelman, 1969; Geisman, 1975; Kim et al., 1979).

Rice is one of the most important food crops in the world. In particular, it is the major diet in the countries of Southeast Asia. However, there is still not sufficient information on the role of washing or cooking processes in removing pesticide residues from rice to assess the safety of consumers' health because of the lack of a satisfactory analytical method.

In this study, an analytical method to determine chlorpyrifos residues spiked in polished rice was developed, and then removal of chlorpyrifos from polished rice under various cooking conditions was investigated. Chlorpyrifos was chosen because it is the active ingredient of a nonsystemic insecticide used widely on a variety of agricultural commodities including rice (Worthing, 1987).

EXPERIMENTAL PROCEDURES

Materials. Authentic chlorypyrifos, 3,5,6-trichloro-2-pyridinol (TCP), and chlorpyrifos oxone were from Dow Chemical Co. (Midland, MI). Silylation reagent bis(trimethylsilyl)acetamide (BSA) was from Aldrich Chemical Co. (Milwaukee, WI). All solvents were of analytical grade and used without further purification. Rice grains (medium-grain variety) were obtained from a local market.

Fortification of Chlorpyrifos to Rice Grains. Polished rice grains (2 kg) were spread over a polyethylene film and then 40 mL of an aqueous chlorpyrifos solution, prepared from a Lorsban 4E formulation (Dow), was evenly sprayed over the rice. The calculated amount of chlorpyrifos fortified was 500 ppb. The fortified rice grains were wrapped with a polyethylene film and stored in a refrigerator at 5 °C for 12 h. They were then dried in an aluminum tray in the shade for 2 days and stored in a freezer at −25 °C until analysis.

Sample Preparations. *Experiment I.* Fortified rice (100 g) was extracted with 600 mL of ethyl acetate in a Soxhlet extractor with a 60 mm × 180 mm thimble at the extraction rate of 60 min/cycle for 24 h. The extract was dried through sodium sulfate placed in a funnel. After an aliquot (100 mL) of the filtrate was concentrated to 20 mL by using a rotary flash evaporator at 40 °C under reduced pressure, the final ethyl acetate solution was analyzed for chlorpyrifos by GC with an NPD. The result is shown in Table I.

Experiment II. Fortified rice (300 g) was washed with a 500-mL portion of tap water three times. After addition of tap water, the rice was swirled with a spoon 10 times and then water was removed by decantation. Volumes of the water removed from the first, second, and third wash were 465, 483, and 498 mL, respectively. The remaining wet rice sample weighed 354 g. Each decantate was mixed with 15 g of Celite 545 and then filtered with a glass microfiber filter backed with a Whatman No. 1 filter paper under reduced pressure. The pesticide residues in the filtrate were trapped by using two Bond Elut CH cartridges (Analytical International Inc., Harbor City, CA) connected in tandem. Cartridges were washed with 5 mL of methanol followed by 5 mL of 0.01 M HCl solution prior to use. The trapped pesticide residues in the cartridges were recovered with 2.8 mL each of ethyl acetate by use of a centrifuge. The combined ethyl acetate solution was analyzed for chlorpyrifos. The results are shown in Table I as "filtrate".

The pesticide residues with Celite 545 were mixed with 200 mL of ethyl acetate and homogenized in a 250-mL Erlenmeyer flask for 1 min by a Tissumizer (Tekmar, Cincinnati, OH). The solid materials were filtered, and the filtrate was adjusted to 200 mL in volume with ethyl acetate. The ethyl acetate solution was analyzed for chlorpyrifos. The results are shown in Table I as "sediment".

Experiment III. The washed rice (118 g) obtained from experiment II was extracted with 500 mL of ethyl acetate by use of a Soxhlet extractor under the same conditions as used for experiment I. The extract was also treated in the same way as for experiment I. The results are shown in Table I.

Experiment IV. The washed rice (236 g) obtained from experiment II was cooked with 240 mL of tap water in a 1000-mL two-neck round-bottom flask and then allowed to stand for 20 min. The flask was connected to a condenser, and steam distillate was collected in a 100-mL receiving flask while the rice was cooking. After 20 min, 435 g of cooked rice and 41 mL of distillate were obtained.

The cooked rice (50 g) was homogenized with 15 g of Celite 545 and 150 mL of acetone in a 300-mL round-bottom flask by use of a Tissumizer for 2 min. After addition of 5 mL of concentrated HCl solution, it was homogenized for an additional 1 min. The Tissumizer probe was washed with 10 mL of acetone. The homogenate was shaken with a Gyrotory shaker for 10 min, and the supernatant was filtered with a glass microfiber filter backed with a Whatman No. 1 filter paper under reduced pressure. The solid materials in the flask were mixed with 100 mL of acetone and homogenized for 1 min and filtered in the same manner as above. The solid residues were washed with 25 mL each of acetone, 2 N HCl, and acetone in sequence. The filtrate and the washes were combined and extracted with 75 and 50 mL of dichloromethane twice. The organic layer was condensed to approximately 50 mL in volume to yield a watery turbid solution. This

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Table I. Amounts of Chlorpyrifos Residues Recovered from Rice Grains after Various Cooking Processes

expt	sample	chlorpyrifos,[a] ppb/raw rice	TCP,[a] ppb/raw rice
I	raw rice	456.0 ± 21.5	3.40 ± 0.43
II	water from washing		
	first wash filtrate	3.1 ± 0.1	0.19[b]
	sediment	174.3 ± 10.8	2.12[b]
	second wash filtrate	1.6 ± 0.1	[c]
	sediment	68.8 ± 7.9	1.11[b]
	third wash filtrate	1.0 ± 0.0	[c]
	sediment	28.7 ± 5.3	0.62[b]
III	washed rice	161.2 ± 2.7	4.50 ± 0.51
IV	cooked rice	130.5 ± 5.4	12.81 ± 1.72
	distillate	11.5 ± 1.0	0.28 ± 0.01

[a] Values are mean ± standard deviation ($n = 3$). [b] $n = 2$. [c] Not detected.

solution was extracted with a 50-mL portion of dichloromethane three times. The extract was dried over anhydrous sodium sulfate. After removal of sodium sulfate, the solution was condensed to dryness. Residual dichloromethane was removed by adding 10 mL of ethyl acetate and evaporated to dryness. This procedure was repeated twice. The residual material was dissolved in 10 mL of ethyl acetate and analyzed for chlorpyrifos. The results are shown in Table I as "cooked rice".

The distillate (41 mL) was extracted with two 75-mL portions of dichloromethane in a separatory funnel. The combined extracts were dried through sodium sulfate placed in a funnel. The extracts were condensed to dryness by using a rotary flash evaporator, and residual material was dissolved into 10 mL of ethyl acetate. This solution was analyzed for chlorpyrifos. The results are shown in Table I as "distillate".

Final ethyl acetate solutions from all samples were condensed to dryness by using a rotary flash evaporator, and the residues were dissolved into 2 mL of hexane. BSA (5 μL) was added to the hexane solutions. The reaction mixtures were analyzed for TCP and chlorpyrifos oxone (possible degradation products of chlorpyrifos) by a GC with a Hall detector set up for halogen mode.

Recovery Studies on Chlorpyrifos from Cooked Rice. Unfortified cooked rice was spiked with 100 μL of an acetone solution of chlorpyrifos (50 μg/mL), which gave a 100 ppb level of chlorpyrifos in cooked rice. The recovery conditions and results are shown in Table II.

Experiment V. One set of cooked rice samples (50 g each) were directly extracted with a solvent (acetone or ethyl acetate) by use of a Soxhlet extractor. The residual cooked rice grains were ground with 150 mL of acetone for 1 min and filtered. The filtrate and the extracts from the Soxhlet extractor were condensed to 50 mL and then extracted with 100 mL of hexane twice. After the hexane solution was condensed to dryness, the residue was dissolved in ethyl acetate for GC analysis. The results are shown in Table II.

Experiment VI. The other set of cooked rice samples (50 g each) were mixed with a solvent (acetone) and then ground with 15 g of Celite 545 by a Tissumizer for 2 min under basic or acidic conditions. After filtration, samples were treated in the same way as for experiment V. Results are shown in Table II.

Qualitative and Quantitative Analysis. Qualitative analysis of chlorpyrifos and TCP was performed by the coinjection method with authentic compounds using a Varian Vista Model 402 controlled Vista Model 6000/6500 GC equipped with dual detectors. Standard curves of chlorpyrifos and TCP were prepared for quantitative analysis (Mourer et al., 1990). For chlorpyrifos analysis, a nitrogen–phosphorus detector (NPD) and a 1.67 m × 2 mm i.d. glass column packed with 5% OV-101 on 60/80 mesh Gas Chrom Q (Applied Science Laboratories, Inc., State College, PA) were used. The helium carrier gas flow was 30 mL/min. The hydrogen and air flow rates were 4.5 and 175 mL/min, respectively. The isothermal oven temperature was 175 °C. The injector and detector temperatures were 220 and 250 °C, respectively.

For TCP analysis, a Tracor Hall Model 700A conductivity detector (halogen mode) and a 25 m × 0.53 mm i.d. methyl silicone bonded phase fused silica capillary column (Perkin-Elmer, Norwalk, CT) were used. The oven temperature was held at 100 °C for 4 min and then programmed to 180 °C at 10 °C/min and held for 14 min. The injector and detector base temperatures were 250 and 300 °C, respectively. The combustion chamber temperature was 900 °C. Electrolyte for the Hall cell was 1-propanal flowing at 0.5 mL/min.

RESULTS AND DISCUSSION

Rice is the major diet in some countries of southeast Asia including Korea and Vietnam as well as Japan and China. Cooking processes for rice vary in different countries, but the most commonly used method is to wash rice grains with water several times and then cook with an appropriate amount of water.

Formation of TCP upon hydrolysis of chlorpyrifos in trace amounts was observed during washing and cooling processes. Other compounds derived from chlorpyrifos, such as chlorpyrifos oxone, were not detected throughout the experiments. Since no TCP was found in unfortified blank rice grains, removal of fortified chlorpyrifos during cooking was calculated on the basis of the total chlorpyrifos and TCP recovered.

From experiment I, the amounts of chlorpyrifos and TCP recovered from raw rice grains were 456.0 ± 21.5 and 3.40 ± 0.43 ppb, respectively. The total recovery as chlorpyrifos (462 ppb) was 92.4% relative to the calculated fortified amount (500 ppb). The calculated fortified amount is, however, not an accurate value because some amount of chlorpyrifos may escape from the system during spraying or storage for equilibration. Therefore, the amount recovered (462 ppb) in this experiment was used as the original quantity of the residue in the raw rice grains.

The results shown in Table I suggest that approximately 60% of fortified chlorpyrifos was removed by washing with water. The results also show that repeated washing decreased residues in rice grains. The washing was repeated three times because that is equivalent to a home-cooking practice. It is interesting that most chlorpyrifos residues were found in the sediment rather than in the supernatant (filtrate) of the rice washes (Table I). It is often observed that rice washes are used to make various soups along with vegetables and soy paste in the rural areas of Asian countries. This practice might be discouraged if raw rice grains were contaminated by pesticide residues.

Table II. Recovery Efficiencies from Cooked Rice Fortified with 100 ppb of Chlorpyrifos by Different Procedures

expt	solvent, mL	acid or base/mL	temp, °C	chlorpyrifos, ppb	TCP, ppb	recovery, %
V	ethyl acetate, 500	a, 0	25	28.0	2.0	30
	acetone, 500	a, 0	25	29.4	1.8	31
VI	acetone, 150	a, 0	25	58.4	3.6	62
	acetone, 150	12 N, HCl, 5	25	72.0	2.0	74
	acetone, 150	12 N HCl, 5	95[b]	58.8	1.2	60
	acetone, 150	10% KOH, 20	25	21.0	16.2	37

[a] No acid or base. [b] For 15 min at this temperature.

Analysis of cooked rice was difficult because it forms a sticky paste. Therefore, various removal methods were examined to find the best recovery of chlorpyrifos from cooked rice (Table II). Direct extraction of cooked rice with an organic solvent with a Soxhlet extractor did not give a satisfactory recovery. It was proposed that a certain amount of chlorpyrifos was bound to rice components such as proteins and carbohydrates. Therefore, samples were acid- or base-treated to release chlorpyrifos from binding. The acid treatment increased recovery at ambient temperature, but at an elevated temperature of 95 °C, compounds were formed which interfered with GC analysis. Identical problems were experienced with base treatment. Therefore, the experiment with cooked rice was conducted under acidic condition at room temperature. The experimental procedure was, however, modified by trial and error several times during the present study and eventually 98 ± 3% recovery was obtained. The method used for cooked rice is described under Experimental Procedures.

During cooking, approximately 7% of the pesticide residues escaped from rice grains in water vapor (distillate in Table I). However, this amount should remain in the cooked rice because most rice grains are cooked in a sealed pot. The cooking process slightly increased TCP residues, which suggests that some chlorpyrifos was hydrolyzed during cooking.

The results of this study indicate that certain amounts of pesticide residues are possibly removed by cooking processes.

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