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Risk Assessment and Degradation of an Insecticide (Chlorpyriphos): A Decontamination Study under Different Culinary Processes in/on Cabbage

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ABSTRACT

Chlorpyriphos 20 EC was applied at the rate of 500 g ai. ha^{-1} to cabbage heads and the samples harvested at intervals of 0 (2 hours after application), 1 and 7 days after application. The calculated half-life value and safe waiting period (8.75 and 45.29 days, respectively) indicated its persistence. Thus, to reduce the safe waiting period, efforts were made to decontaminate the chlorpyriphos residue from the cabbage head by various household preparations (viz. washing, cooking, washing plus cooking, salt water dipping, dipping in boiled salt water, dipping in detergent solution and dipping in boiled detergent solution). Statistical analysis of the data using Duncan's multiple range test revealed that various household processing treatments substantially reduced the residue of chlorpyriphos in cabbage heads by a range of 27.89-73.32% but none were able to satisfactorily reduce the residue to below the tolerance level of 0.05 mg kg^{-1}.

Key words: chlorpyriphos, residues, decontamination, cabbage, household preparations

INTRODUCTION

Cabbage (*Brassica oleracea* var. capitata) is an important winter vegetable crop grown in India that is heavily attacked by many pests, including the diamond-back moth (*Plutella xylostella*), leaf eating caterpillars and aphids, resulting in severe loss of quality and production (Regupathy *et al.*, 1985; Patel *et al.,* 1999). Chlorpyriphos (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) is a contact insecticide intensively used by farmers in many parts of West Bengal as a plant protection measure.

Chlorpyriphos toxicity to birds ranges from moderate to very high. Its oral LD50 is 8.41 mg/kg in pheasants, 112 mg/kg in mallard ducks, 21.0 mg/kg in house sparrows and 32 mg/kg in chickens. The LD50 for a granular product (15G) in bobwhite quail was 108 mg/kg (Kidd and James, 1991; USPHS, 1995). At 125 ppm, mallards laid a significantly smaller number of eggs. There was no evidence of changes in weight gain, or in the number, weight and quality of eggs produced by hens fed dietary levels of 50 ppm of chlorpyriphos (USPHS, 1995). In addition, chlorpyriphos is very highly toxic to freshwater fish, aquatic

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invertebrates and estuarine and marine organisms. Cholinesterase inhibition was observed in acute toxicity tests of fish exposed to very low concentrations of this insecticide. Applications of concentrations as low as 0.01 pounds of active ingredient per acre may cause the deaths of fish and aquatic invertebrates (USEPA, 1989). Chlorpyriphos toxicity to fish may be related to water temperature. The 96-hour LC50 for chlorpyriphos is 0.009 mg/L in mature rainbow trout, 0.098 mg/L in lake trout, 0.806 mg/L in goldfish, 0.01 mg/L in bluegill, and 0.331 mg/L in fathead minnow (USEPA, 1986). When fathead minnows were exposed to Dursban for a 200-day period during which they reproduced, the first generation of offspring showed decreased survival and growth rates, as well as a significant number of deformities. This occurred at approximately 0.002 mg/L exposure for a 30-day period. Furthermore, chlorpyriphos accumulates in the tissues of aquatic organisms. Studies involving continuous exposure of fish during the embryonic through to the fry stages have shown bioconcentration values of 58 to 5100 (Racke, 1992). Due to its high acute toxicity and its persistence in sediments, chlorpyriphos may represent a hazard to sea-bottom dwellers (Schimmel, 1983). Smaller organisms appear to be more sensitive than larger ones (USEPA, 1986). Aquatic and general agricultural uses of chlorpyriphos pose a serious hazard to wildlife and honeybees (USEPA, 1984).

Surveys conducted in India have indicated that 50-70% of vegetables are contaminated with insecticide residues (Kole *et al.,* 2000; Karanth, 2000), probably due to their use in the field not in accordance with good agricultural practices and in particular harvesting the crops before the end of the recommended waiting period. In general, pesticide analyses are performed on raw agricultural commodities that include the peel and (other) non-edible parts. However, cabbages are subjected to some form of household preparation before actual consumption by, for example, washing, cooking or the removal of non-edible parts. Some studies have shown that certain types of postharvest treatment or household preparation may help to reduce pesticide residues (Ramesh *et al.,* 1999; Krol *et al.,* 2000; Zabik *et al.,* 2000). The effects of these processing techniques on residue levels are extremely important in evaluating the risk associated with ingestion of pesticide residues. To date, very little information has been available on the influence of food processing on a specific pesticide-commodity combination and this is important because the behavior and fate of the chemical varies with the pesticide as well as with the crop. The data regarding the effect of various household preparations on the reduction of chlorpyriphos residues in cabbage is scanty. If the insecticide persisted in the harvested or mature plant parts or even the leftover part before consumption, it would increase the pollutant load in the environment. Thus, it may cause serious damage to the ecosystem, particularly to various beneficial organisms and predators of pests. If the insecticide is present in the consumable part of the crop, then it will cause direct damage to consumers. Therefore, the present investigation was carried out with the objective of examining the persistence of chlorpyriphos on cabbage heads and to evaluate the impact of various household preparations (washing, cooking, washing plus cooking, salt-water washing, detergent washing) on the reduction of chlorpyriphos residues in cabbage heads.

MATERIALS AND METHODS

Field experiment and collection of samples

The experiment was conducted in cabbages (variety Indian Rare Ball) at the Agricultural Research Farm, Baruipur under the operational control of the Institute of Agricultural Science, University of Calcutta, Kolkata, West

Bengal, India, during September to December 2005. The climatic parameters for the season at the experimental the site were: temperature 21.04-27.50°C; relative humidity 86.71%; rainfall 164 mm; and wind strength normal (8-10 km h^{-1}). Chlorpyriphos 20 EC was purchased from the local market and applied at the rate of 500 g ai. ha^{-1} halfway through the fruiting stage. The formulation was diluted with water and sprayed at 600 L ha^{-1} with a knapsack spray. Samples of cabbage heads (~20 kg) were drawn randomly from the whole field (90 m^2) at 0 (2 h after application), 1 and 7 days after spraying. The samples were packed into brown paper bags and then brought to the laboratory, cut into small pieces, mixed thoroughly and sub-samples (3 × 100 g) of fresh heads were weighed for each household processing treatment.

Household preparation

In the first treatment, each replicated sample (100 g) was washed under running tap water for 2 min (T_1). In the second treatment, the heads were cooked in boiling water (500 ml for each 100 g sample) for 5 min and the water was discarded (T_2). The third treatment was a combination of the first two, i.e. heads (100 g) were washed thoroughly under tap water for 2 min followed by boiling in 500 ml water for 5 min and the water was discarded (T_3). In the fourth and fifth treatments, the heads were dipped in 500 ml of 2% salt brine solution at room temperature, (28±1°C; T_4) and under hot (85±1°C; T_5) conditions for 5 min and then washed under tap water for 2 min. In the sixth and seventh treatments, this procedure was applied using 1% detergent solution (pH 10.2) at room temperature (T_6) and under hot conditions (T_7) for 2 min followed by washing for 5 min. The field samples analyzed without any additional household technique were designated as the unprocessed control (T_0).

Extraction and clean-up of residues

The unwashed and the various home-processed cabbage samples were blended separately in a Remi–Automix blender for two min with 150 ml acetone. Each sample was then filtered, concentrated (~20 mL) using a rotary vacuum evaporator, partitioned three times with dichloromethane (100+50+50 mL), combined and concentrated to ~ 10 mL. The organic phase was further cleaned up using a C-18 silica gel Solid Phase Extraction (SPE) column. The SPE column was eluted with 200 ml ethyl acetate, evaporated to dryness, and the volume made up with distilled hexane (10 ml) for gas chromatographic analysis.

Estimation of residues

An aliquot (1ml) of cleaned-up extract was injected into a gas chromatograph with a 10 ml Hamilton Syringe. The residue of chlorpyriphos in each sample was identified by comparing the retention time of the sample peaks with the standard (99.5% purity, Sigma-Aldrich) solution containing 1 ppm of chlorpyriphos. The residues of chlorpyriphos were analyzed on a GC (Agilent Technologies 6890N Network GC system) with an electron capture detector (ECD-Source Ni^{63}) coupled with a Chemito 5000 data processor. An HP-5 capillary column (30m × 0.32mm i.d.) of 0.25 μm film thickness was used. The temperature for the oven, injector and detector was set at 210, 230 and 300°C, respectively. The flow rate of the carrier gas was 2 mL min^{-1}. The retention time, limit of detection (LOD) and limit of quantification (LOQ) were 5.38 min, 0.01 μg g^{-1} and 0.05 μg g^{-1}, respectively.

Recovery studies

In order to estimate the efficiency of the method, a recovery experiment was conducted by fortifying untreated samples with analytical grade chlorpyriphos (99.5% purity, Sigma-Aldrich) at the rates of 0.25, 0.50 and 1.00 μg g^{-1}. The fortified samples were analyzed and estimated following

the method described in the previous section. Recovery amounts from this method were in the range of 93-98% with an average of 95.67% (Table 1). The limit of quantification of the method was 0.01 mg kg^{-1}.

Statistical analysis

The residue data were subjected to statistical analysis for the computation of regression equations and half-life ($T_{1/2}$) values. The $T_{1/2}$ values thus obtained were further tested by one-way analysis of variance (ANOVA) using the SPSS 10.0 statistical package. Using the same package, Duncan's multiple range test (DMRT) (at $p < 0.05$) was calculated to judge the statistical significance of the various home processing treatments with regard to reducing chlorpyriphos residues.

Dietary risk assessment of chlorpyriphos through cabbage consumption

The dietary intake of chlorpyriphos and the acceptable daily intake of chlorpyriphos were determined using Equations 1 and 2, respectively:

$$\text{Dietary intake} = \frac{\sum(R_i \times F_i)}{BW} \quad (1)$$

$$\%\ \text{ADI} = \frac{\text{Dietary intake} \times 100}{\text{ADI}} \quad (2)$$

where:

R = Residues of chlorpyriphos in μg g^{-1} in Cabbage

F = Food (cabbage) consumption ≅ 10g

BW = Average body weight = 55kg

ADI = Acceptable daily intake of chlorpyriphos (0.01 mg/kg body weight)

RESULTS AND DISCUSSION

Persistence of chlorpyriphos

The initial residue of chlorpyriphos in the cabbage heads after 2 h (0 day) of spray was 2.91 mg kg^{-1} (Table 2). After 1 and 7 days, residues declined to 2.45 and 1.61 mg kg^{-1}, respectively, showing a reduction of 15.81and 44.67%, respectively. The dissipation of chlorpyriphos residues followed first order reaction kinetics. The calculated half–life ($T_{\circ}$) value was found to be 8.75 days, indicating the persistent nature of chlorpyriphos. The low temperature in the winter season might have led to the slow dissipation of the chlorpyriphos residues in the cabbage heads.

Effect of various household preparations

Washing the cabbage head under running tap water (T_1) removed an average 27.89% of the chlorpyriphos residues from head (Table 2). After cooking (T_2), the reduction was 41.40% and with washing plus cooking (T_3) it further improved to 66.78%. This seemed to suggest that loosely adhering surface residue was removed by the water wash, while the more inaccessible amounts of chlorpyriphos that may have penetrated into the surface of the heads might not have been

Table 1 Results of method validation by recovery analysis of chlorpyriphos (analytical grade) from cabbage heads.

Substrates	Amount fortified (μg g^{-1})§	Amount recovered (μg g^{-1}) §	Recovery (%)	Average recovery (%)
Cabbage heads	0.25	0.23	93	
	0.50	0.48	96	95.67
	1.00	0.98	98	

§ Average of three replicates.

appreciably reduced by cooking for 5 min in boiling water. It was also observed that separate treatments of washing and cooking (T_1 +T_2) reduced a total of 69.29% chlorpyriphos residues, close to the 66.78% reduction when subjected to washing and cooking in succession (T_3). The half-life values of chlorpyriphos reduced from 8.75 to 3.85 days under home processing treatment T_3 and this value was significantly lower than T_1 and/or T_2 at the 5% level of significance (Table 2). Thus, washing or cooking alone did not help much in reducing the chlorpyriphos residues, compared to the washing treatment followed by cooking. A minimum of about twelve days was suggested as a safe waiting period.

Dipping in 2% brine solution (T_4) followed by washing reduced the residues by 39.58%, while in the case of the hot 2% brine solution (T_5) this reduction was 55.01%. However, T_4 did not differ significantly from T_1 or T_2. Similar observations with malathion, quinalphos and chlorpyriphos in cabbages were also reported by other authors (Jacob and Verma, 1989; Jacob and Verma, 1991; Nagesh and Verma, 1997).

Dipping in 1% detergent (T_6 and T_7) followed by thorough washing reduced the residues by 73.32 and 71.18%, respectively, thereby showing the effectiveness of a detergent wash in removing chlorpyriphos, which was 2.6 times more efficient compared to a water wash. The hydrolysis of chlorpyriphos occurred readily at pH > 7 (Figure 1), (Smith, 1968) which might arise due to the detergent solution, resulting in the higher removal of chlorpyriphos. The degradation of chlorpyriphos with time under different culinary processes is presented in Figure 2. The effectiveness of the different treatments on $T_{1/2}$ was in the order of $T_3 \approx T_7 > T_6 \approx T_5 > T_4 \approx T_1 \approx T_2 \approx T_0$ (Table 2).

The dietary risk assessment of chlorpyriphos (Table 3) revealed there was no appreciable risk arising through cabbage consumption. The dietary intake of chlorpyriphos was in the range of 0.05–0.50 μg kg^{-1} body weight over 0, 1 and 7 days contributing only a meager 1.58–5.01% of ADI (0.01 mg kg^{-1}) based on the 0 day sample data. The % contribution was further decreased to 1.01–4.23 and 0.46–2.78% based on the 1 and 7 days sampling, respectively, regardless of the type of household treatment followed. Although the consumption of cabbage alone does not represent any risk to consumers, considering the wide range of chlorpyriphos used on various crops (Rengasamy and Dureja, 2001), this may mean that cabbage could contribute a significant amount to the chlorpyriphos intake in the total diet.

Table 2 Residues of chlorpyriphos in cabbage heads and its removal by household preparations.

Treatments	Residues in mg/kg * (±SD) at different days interval			Mean % reduction	Mean $T_{1/2}$ days**
	0	1	7		
T_0	2.91 (± 0.02)	2.45 (± 0.03)	1.61 (± 0.05)	-	8.75 [a]
T_1	2.39 (± 0.01)	1.49 (± 0.01)	1.19 (± 0.01)	27.89	8.85 [a]
T_2	1.94 (± 0.03)	1.19 (± 0.16)	0.98 (± 0.04)	41.40	9.29 [a]
T_3	1.59 (± 0.08)	0.60 (± 0.03)	0.33 (± 0.01)	66.78	3.85 [c]
T_4	1.79 (± 0.04)	1.47 (± 0.04)	0.96 (± 0.04)	39.58	8.46 [a]
T_5	1.74 (± 0.03)	0.84 (± 0.02)	0.66 (± 0.07)	55.01	6.73 [b]
T_6	0.92 (± 0.05)	0.63 (± 0.06)	0.36 (± 0.01)	73.32	5.85 [b]
T_7	1.34 (± 0.02)	0.58 (± 0.07)	0.27 (± 0.03)	71.18	3.57 [c]

* Average of three replications

** Similar letters signify homogeneous means due to DMRT.

Table 3 Dietary exposure of chlorpyriphos through consumption of cabbage heads.

Treatments	Dietary intake (DI, mg/kg body weight) and % ADI of chlorpyriphos on different days							
	0		1		7		Mean	
	DI	% ADI	DI	% ADI	DI	% ADI	DI	% ADI
T_0	0.50	5.01	0.42	4.23	0.28	2.78	0.45	4.51
T_1	0.41	4.12	0.26	2.56	0.20	2.04	0.33	3.27
T_2	0.33	3.34	0.20	2.04	0.17	1.69	0.27	2.65
T_3	0.27	2.74	0.10	1.04	0.06	0.57	0.16	1.63
T_4	0.31	3.08	0.25	2.53	0.17	1.66	0.27	2.73
T_5	0.30	2.99	0.14	1.44	0.11	1.14	0.21	2.09
T_6	0.16	1.58	0.11	1.09	0.06	0.63	0.12	1.24
T_7	0.23	2.30	0.10	1.01	0.05	0.46	0.14	1.42

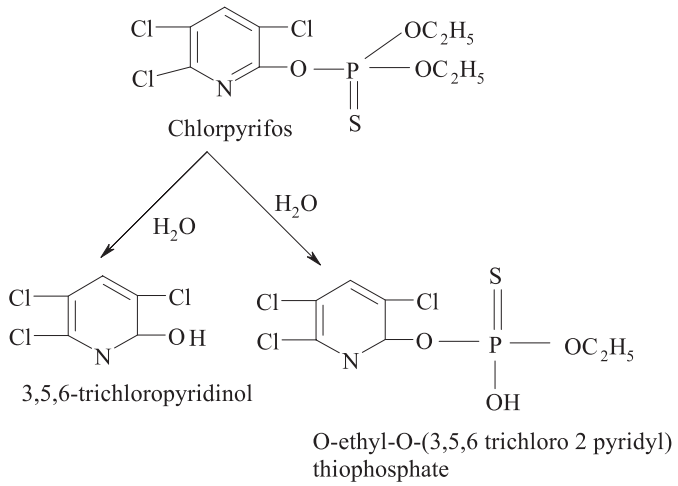

Figure 1 Probable degradation of chlorpyriphos at pH > 7.0.

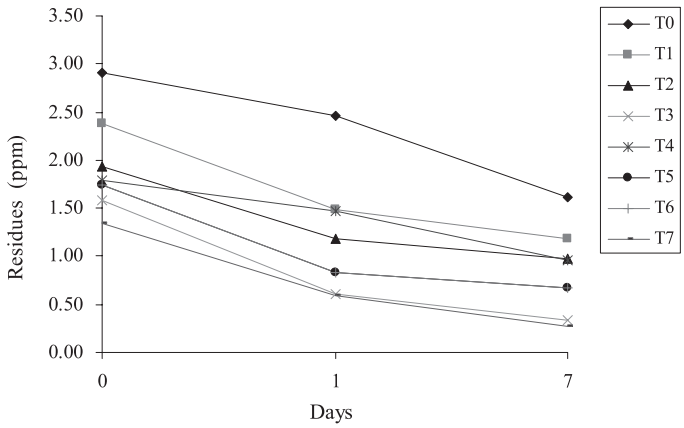

Figure 2 Degradation of chlorpyriphos under different culinary processes.

CONCLUSIONS

A comparison of the overall effects of different household preparations indicated that the level of chlorpyriphos residue could be reduced significantly by mild detergent washing or by washing plus cooking. The reduction in residue levels made these procedures worthwhile for adoption by the consumer. The strong adsorption properties coupled with poor water solubility of chlorpyriphos might have been responsible for reducing the efficiency of the home processes for decontaminating the cabbage heads. Hence, to reduce the risk associated with the intake of chlorpyriphos through cabbage heads, washing plus cooking or mild detergent washing procedures should be followed before consumption.

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