

## CHLORPYRIFOS DEGRADATION IN SOIL AND ITS EFFECT ON SOIL MICROORGANISMS

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### ABSTRACT

This study was designed to determine the degradation of chlorpyrifos, an organophosphate (OP) insecticide used now a day for termite control in replacement of heptachlor. Beside this it is also used on agricultural crops for pest control. Soil samples from farmer field at Memon Goth Karachi were collected randomly from a depth of 0-25cm. The persistence was determined at 100 ppm and 1000 ppm in soil as well as in combination with different fertilizers @ 1% viz: (Foliar fertilizer Polydol, DAP, SOP, and Urea) with tap water used for drinking purposes as well as hard water used for irrigation. The treated samples were kept under darkness to maintain the microbial activity and later analyzed through GC for persistence. The result of six months and one year study showed that no degradation occurred during the study period i.e. 100% recovery of the active ingredient was observed after one year under lab conditions. The microbial activity was also monitored by the standard dilution pour plate technique. The growth of *Bacillus sp.* was sensitive to chlorpyrifos treatment i.e. number of colony decreased whereas number of colonies of *Klebsiella sp.* was found enhanced during the same treatment in the experiments.

**Key words:** Chlorpyrifos, degradation, management, persistence, pesticide.

### INTRODUCTION

Application of the pesticide on agricultural crop is now a common practice and is an important factor of integrated pest management (IPM) strategies. It adversely affects the properties of the soil as well as it alters the pH of the soil required for microbial activities of beneficial bacteria to act upon. (Rahman and Motoyama, 2000; Malinowski, 2000)

The maintenance of soil biodiversity is very important for substantial management of soil properties by minimizing the risks of soil and environmental degradation (Lal, 1999). Due to environmental significance of pesticides and their residues, a thorough understanding of the physical, biological and chemical forces acting upon these chemicals is important (Xuang *et al.*, 2000). Approximately less than 0.1% of applied pesticide reaches the target pest, leaving the bulk to affect the environment (Ardley, 1999). One of the main environmental concerns with pesticides is their potential to affect soil, which is controlled primarily by their persistence and mobility in the soil (Walker, 2003). The most important factors affecting the activity of the insecticides are: the mechanism of action of the active ingredient, the defense mechanism of the treated insects, the type of formulation, its systemic properties, mode of application, the moisture content and temperature of soil (Rehman and Motoyama, 2000). It has been reported that repeated application of chlorpyrifos to the soil did not result in the development of a microbial population with

the enhanced ability to degrade the pesticide (Singh *et al.*, 2003).

Organophosphates (OP) are generally regarded as safe for use on crops due to their relatively fast degradation rate, which varies as a function of microbial components, pH, temperature, hydrolysis, photolysis and other factors. Hydrolysis half-life of an OP pesticide of 10 days in the lab increases to one year if the pH is 6 and temperature is 5°C (Ragnarsdottir, 2000). In one of the studies organic carbon content and relatively low pH of the soil greatly affected the released residue of chlorpyrifos. Sterilization greatly reduced the rate of chlorpyrifos degradation indicating the involvement of microbial activities (Rehman and Motoyama, 2000). Chlorpyrifos incubated for 69 days at 15°C showed that soil pH was strongly co-regionalized with chlorpyrifos residue (Price *et al.* 2003). It has been observed that chlorpyrifos had insignificant effect on soil microorganism (Singh *et al.* 2002a). The half-life of chlorpyrifos was 34-46 days with negligible effect on microbial characteristics (Singh *et al.*, 2002b). In a 2 months study 100% persistent was observed with the survival of *Klebsiella sp.* (Shahida *et al.*, 2004). Chlorpyrifos has been reported previously to be resistant to enhanced degradation due to the accumulation in soil of the antimicrobial degradation product. Its microbial degradation results in higher concentration of 3,5,6, trichloro-2-pyridinol (TCP), a major metabolite (Robertson *et al.*, 1998). According to one report, chlorpyrifos residues in soil had a temporary or short

term inhibitory effect on soil microbial functional diversity (Hua Fanga *et al.*, 2009).

Chlorpyrifos is one of the most widely and commonly used commercial insecticides (Kuperberg *et al.*, 2000). Considering its persistence up to 2 months from the recently conducted study (Shahida *et al.*, 2004), this study was initiated to determine the degradation of chlorpyrifos in combination with fertilizers viz. DAP, SOP, Urea and foliar fertilizer Polydol up to one year under lab conditions using hard and tap water.

## MATERIALS AND METHODS

**Collection of soil:** Vegetable growing fields in Memon Goth, Karachi were selected for this study and soil was collected randomly from 0-25cm depth from four corners 4-6 meters part and from centre of the field. The samples were pooled together, brought to the laboratory in polyethylene bags and kept in refrigerator at 5-6°C to maintain the biological activity of the soil microbes and analyzed at pesticide research lab Southern Agricultural Research Centre (SARC) Karachi.

**Soil treatment incubation:** The organophosphates pesticide Chlorpyrifos [Kurifast 40% EC] used at concentration of 100 ppm and 1000 ppm and fertilizers (Polydol, DAP, SOP & Urea) at concentration of 1% each were mixed with 25 g of soil already dried and sterilized in glass tubes. The autoclaved soil and autoclaved water (Tap/Hard) was used as control. Source of fertilizer Polydol was from Pak Agro Chemicals and other DAP, SOP and Urea were purchased from local market. The treatments made were as follow;

- T1- Soil + Pesticide
- T2- Soil + Pesticide + Culture
- T3- Soil + Pesticide + Culture + DAP
- T4- Soil + Pesticide + Culture + SOP
- T5- Soil + Pesticide + Culture + Urea
- T6- Soil + Pesticide + Culture + Polydol

One gram of soil was added in 100 g of sterilized water for the preparation of soil culture. Individual treatments of soil in triplicate were flooded with tap/hard water to keep them moist during incubation period. These treatments were kept at room temperature (28-30 °C) under darkness for 6 and 12 months. Two sets of tubes were prepared with the pesticides at the rate of @100 and 1000 ppm. After completion of incubation period tubes were taken out accordingly for extraction and analysis.

**Extraction and analysis:** Twenty five gram of treated soil (samples) were air dried for extraction and homogenized with 0.5 g charcoal (activated) for 4 hrs at 120°C. Then added 1.0 g Florisil (activated for 4 hours at 650°C) and 5 drops of 25% NH<sub>4</sub>OH solution, placed over a 2.5 cm layer of anhydrous sodium sulphate (analytical grade) in a glass column with 34 cm length and 2.5 cm

dia. Extraction was done by using a solution of n-hexane (distilled) and acetone (distilled) in a ratio of 2:1 by the method as described by Mumtaz *et al.*, (1983). Eluted material was collected in a 250 ml conical flask (Pyrex) and later evaporated on rotary evaporator to almost dryness dissolved in 2-5 ml quantity n-hexane in small glass vials for GLC determination.

**Gas chromatographic determination:** The extract was analyzed on GC (Varion-3600) USA (equipped with flame ionization detector) with the parameters as column temperature 230°C, injector temperature 250°C, detector temperature 300°C, hydrogen gas flow 4.5 ml/min and air flow 175 ml/min. The parameter of the glass column was 1.5 % OV-17 + 1.75 % OV-210 Chrom W-HP 80/100 mesh 2 meters x ¼" x 2 mm ID (internal diameter). The retention time was 1.6 min., detection limit was 1µ gm and recovery percentage was 100 %.

**Microbial growth activity:** Aliquot samples (approximately 1 g) of soil from each treatment were taken to monitor the microbial activity by the standard dilution plate technique (using nutrient agar medium) as described earlier (Shahida *et al.* 2004). Briefly, the plates were incubated at room temperature (26°C) for 24 hours. Colonies were observed and counted. Later on the colonies were picked up and transferred to nutrient broth tubes for growth and further identification.

**pH observation:** The change in pH with (tap/hard water) fertilizer and pesticide individually without soil was measured after 0, 24 and 48 hours. Similarly the pH changes in tap and hard water after adding soil, fertilizers and pesticides in combination was also measured after 2, 6 and 12 months.

## RESULTS AND DISCUSSION

The data recorded after 6 months and 12 months for chlorpyrifos belonging to organo-phosphorus group, showed that it remained stable even after one year in the soil. No degradation was found in the pesticide as we obtained 100 % recovery of the active ingredient in all the six treatments kept up to one year. Chromatogram of the pesticide at different time period has been shown in Fig.1. Similar results have been reported earlier on this group of pesticide that showed 100% persistency (Shahida *et al.*, 2004). Wright *et al.* (1991) reported the persistency of this insecticide used for the control of termite even after four years. Simcox *et al.* (1995) detected chlorpyrifos in dust samples from agricultural and non-agricultural household even in very low concentration of 17ppb. However, our results are in agreement with the finding of Chelsea (1991) i.e. Chlorpyrifos is moderately persistent in soils with the half-life usually between 60 and 120 days, but can range

from 2 weeks to over 1 year, depending on the soil type, climate, and other conditions.

The changes in pH of the tap and hard water by adding pesticide and fertilizers without soil was recorded and given in Table 1. In case of autoclaved soil and pesticide combination, the pH slightly rose from neutral (7) to alkaline and remained as such till one year for both tap and hard water. But the pH remained neutral or slightly acidic in combination of soil +pesticide+ culture for both treatments of tap and hard water. But when DAP was added to the combination the pH remain constant at neutral for tap water while it was slightly acidic side for hard water treatment. However in case of SOP addition, it was on alkaline side (pH 8) for tap water and on neutral side for hard water (pH 7). When Urea was added to the combination of soil + pesticide + culture, the pH of tap water increased (pH 9) till two months but slightly decreased to pH 8 later on. While in case of hard water treatment, the pH increased from pH 7 to 8. In case of Polydol combination the pH of both tap and hard water remained constant i.e. neutral (pH 7).

The study showed that slight change in pH had no correlation with the persistence of pesticide as we obtained 100% recovery after 1 month, 2 months, 6 months and 12 months incubation under lab conditions (Figure 1). The highest dose of chlorpyrifos applied in our study was near to the termite control rate (1000 µg g<sup>-1</sup>) as reported by Wright *et al.* (1991) in which they measured soil chlorpyrifos along the foundations distributions for indoor of households that have been treated for termites control for 4 years earlier. Similarly, Simcox *et al.* (1995) collected dust samples from agricultural and non agricultural households, compared to the soil samples; chlorpyrifos in dust was nearly ubiquitous, detected in 9 of 11 non-agricultural households and 47 of 48 agricultural households with 17 ppb. Chlorpyrifos levels in the home are expected to be highly dependent on the extent to which applications have been made in the household.

The study on microbial growth during the experiment showed that 100 ppm fortification did not affect adversely but 1000 ppm application suppressed the growth of certain colonies while count of some of the

colonies was increased. Initially two types of colonies were observed in the culture identified as *Bacillus sp.*, and *Klebsiella sp.*, but after incubation for 6 months and 1 year, only one type of colony which was pin pointed and small in size identified as *Klebsiella sp* was found prevalent in both tap and hard water formulations (Table 3). It was found that *Bacillus sp* was sensitive to chlorpyrifos application whereas *Klebsiella sp* which is also a nitrogen fixing bacteria was found to be enhanced in all the treatments except in the treatment having autoclaved soil where there is no growth of any other type of micro organism on nutrient agar medium. Singh *et al.* (2003) reported a robust bacterial population that utilized chlorpyrifos as a source of carbon in an Australian soil. It has been reported that microbial degradation results in higher concentration of TCP (Robertson *et al.*, 1998). Hua Fanga *et al.*, (2009) reported that chlorpyrifos residues in soil had a somewhat temporary or short term inhibitory effect on soil microbial activity.

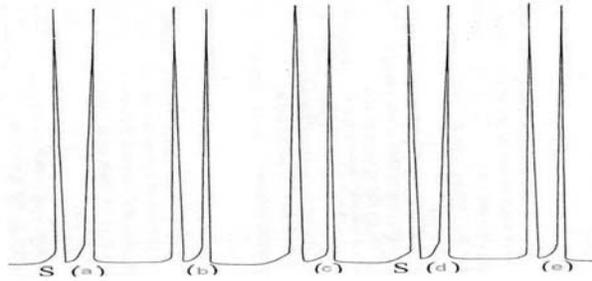
The results of this study indicated that chlorpyrifos remained stable in soil for one year and does not correlate with the pH change occur during study period as no significant change was observed on the recovery of active ingredient of the chemical. These studies suggested that further experiments need to be conducted to determine the time period for degradation of chlorpyrifos in soil.

**Table 1: pH changes in tap and hard water after adding fertilizers and pesticides with out soil.**

Treatments	Tap water			Hard water		
	0 hr	24 hr	48 hr	0 hr	24 hr	48 hr
Water	7	9	9	7	8	8
Water+ SOP	8	10	10	7	8	9
Water+DAP	7	7	7	7	6	5
Water + Urea	7	9	9	7	7	8
Water+ Polydol	7	7	7	7	6	6
Water+ Kurifast	8	9	9	6	8	7

**Table 2: pH of tap and hard water after adding soil, fertilizers and pesticides individually after 0 hour, 2 months, 6 months and 1 year.**

Treatments	Tap Water				Hard Water			
	0 hour	2 months	6 months	1 year	0 hour	2 months	6 months	1 year
Soil (Autoclaved) + Pesticide	7	8	7	7	7	7	6	7
Soil + Pesticide + Culture	7	8	7	6	6	6	6	6
Soil + Pesticide + Culture + DAP	7	7	7	6	7	6	6	6
Soil + Pesticide + Culture + SOP	7	9	8	8	7	7	6	7
Soil + Pesticide + Culture + Urea	7	9	8	7	6	7	6	8
Soil + Pesticide + Culture + Polydol	7	8	7	7	7	7	6	7



**Figure 1:** Gas chromatograms of chlorpyrifos (a-e) extracted from treated soil a (0 day) b (1 month) c (2 months) d (6 months) e (1 year) & S (solvent).

**Table 3:** Microbial growth of *Klebsiella* sp colonies on agar plates after 2, 6 & 12 months.

Treatments	2 Months		6 Months		12 Months	
	H.W	T.W	H.W	T.W	H.W	T.W
Soil (Autoclaved) + Pesticide	-	-	-	-	-	-
Soil+Pesticide+Culture	+	+	+	+	+	+
Soil+Pesticide+Culture+DAP	+	+	+	+	+	+
Soil+Pesticide+Culture+SOP	+	+	+	+	+	+
Soil+Pesticide+Culture+Urea	+	+	+	+	+	+
Soil+Pesticide+Culture+Polydol	+	+	+	+	+	+

TW = Tap water ; HW = Hard water

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