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Original Research Article

Photolysis of Chlorpyrifos in Water under Direct Sunlight - Identification of Photo-transformation products by LC-MS-MS Electro spray Tandem Mass Spectrometry

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Abstract: The photolysis of Chlorpyrifos technical was assessed by following the OECD guideline 316. The Tier 1: Theoretical screen of photolysis test for Chlorpyrifos technical was conducted to determine the approximate half-life by taking a single UV-Visible absorption spectrum for Chlorpyrifos technical in sterile pH 4 buffer solutions from 250nm to 800nm. Based on tier 1- theoretical screen study approximate half-life value obtained less than 30 days. The tier 2- experimental study was conducted at 25± 2°C under direct Sunlight. The percent degradation, half-life and rate constant data calculated following Tier 2 study. Based on the Tier 2 study the results, the direct photolysis rate constant (Kd) of Chlorpyrifos technical was 0.07119 day-1 and Half-life (t1/2) value was 9.73 days. The fortified samples showed an average recovery of 96.88% in pH 4 buffer solution. LC-MS analysis was done to identify the degradation product of Chlorpyrifos Technical in both, irradiated and non-irradiated samples on day 0, 3, 7, 10, 15, 20, and 30. The degradation products identified in the irradiated samples are O, O-diethyl hydrogen phosphorothioate, O-(amino methyl) O, O-diethyl phosphorothioate, and O-ethyl O-methyl O-(3, 5, 6-trichloropyridin-2-yl) phosphorothioate on day 3, day 7, and day 10 in Chlorpyrifos Technical. No degradation product was identified in the dark control Chlorpyrifos Technical samples. On the basis of these results, aqueous abiotic photolysis is expected to contribute significantly to the degradation of Chlorpyrifos technical. Direct photolysis is expected to be a major route of degradation of Chlorpyrifos in the environment as evidenced by the absorbance of Chlorpyrifos in UV/VISIBLE at the wavelength > 290 nm.
Keywords: Chlorpyrifos, Photolysis, Half-life, LC-ESI-MS/MS, UV-Visible absorption.

INTRODUCTION

Photo Chemistry is one of the main routes for organic pollutants attenuation in surface waters. This abiotic degradation pathway has received increasing interest in the last thirty To predict the fate of pollutants in the natural environment and to access the risk they may pose, it is essential to improve our knowledge on their chemical reactions. Several types of reactions might also occur relying at the medium composition [1, 2]. Direct Photolysis is feasible if the taken into consideration pollutant absorbs solar light. Similarly, photo induced or photosensitized changes mediated by means of additives of the aquatic medium can also take location. in particular, dissolved natural organic matter (DOM) which absorbs a large part of photons is potential photosensitizer [3]. Singlet oxygen, superoxide ion/ Hydroperoxyl radicals, hydroxyl radicals, excited triplet states and alkylperoxyl radicals were proved or proposed to be generated in natural waters under the impact of sunlight However, part of these species are trapped by means of DOM itself. Contamination of floor water due to the extensive use of crop protection chemical substances in agriculture is a main public health threat problem. Pesticide residues are likely to get transferred to aquatic reservoirs by way of ground runoff and leaching [4, 5]. Photograph-degradation is one of the crucial elements contributing to the fast dissipation of agrochemicals in water. Chlorpyrifos is a broad-spectrum organophosphate insecticide. Whilst originally used on the primarily to kill mosquitoes, it is no longer registered for this use. Chlorpyrifos is effective in controlling cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, and lice [6, 7]. It's far used as an insecticide on grain, cotton, field, fruit, nut and vegetable crops, and properly as on lawns and ornamental plants. It's also registered for direct use on sheep and turkeys, for horse site treatment, canine kennels, domestic dwellings, farm buildings, garage containers, and industrial institutions. Chlorpyrifos acts

on pests primarily as a contact poison, with some movement as a stomach poison. It's available as granules, wettable powder, dustable powder and emulsifiable concentrate. Chlorpyrifos adsorbs strongly to soil particles and it is not readily soluble in water [8]. It is therefore immobile in soils and unlikely to leach or to contaminate groundwater. It is not mobile in sandy loam and loamy sand soils. TCP, the precept metabolite of chlorpyrifos, adsorbs weakly to soil particles and looks to be moderately mobile and persistent in soils [9]. Chlorpyrifos enters freshwater and saltwater ecosystems primarily as spray drift. It is also carried on eroded soil particles from treated areas. If soil with adsorbed chlorpyrifos is carried by runoff, surface water may be contaminated [10]. In water, chlorpyrifos readily adsorbs to suspended sediment and bottom materials. Volatilization is probably the primary route of loss of chlorpyrifos from water. Volatility half-lives of 3.5 and 20 days have been estimated for pond water. The photolysis half-life of chlorpyrifos is 3 to 4 weeks during midsummer in the U.S., but photo degradation of chlorpyrifos is not expected to be significant in deep waters, during winter, or in waters which sunlight cannot penetrate. Its change into other natural forms (biotransformation) is slow [11]. Research shows that this insecticide is risky in water, and the price at which it's far hydrolyzed increases with temperature, decreasing by way of 2.5 to three-fold with each 10° C drop in temperature [12]. Rate of hydrolysis is consistent in acidic to neutral waters, but increases in alkaline waters. In water at pH 7.0 and 25 ° C, it had a half-life of 35 to 78 days. The half-life of chlorpyrifos in water of an unknown pH was about 80-100 days. The literature review clearly indicates extensive studies are conducted using this insecticide in soil to understand the dissipation, mobility and run off. Direct photolysis is one of the reasons for the attenuation of numerous organic pollutants in surface water. The kind of reactions that a compound undergoes relies upon the medium and the components ensuing within the formation of foremost photoproducts [13]. Those products can also likely to pose threat to human health and environment. The investigations presented in this examine had been centered to understand the dissipation behavior of chlorpyrifos completely in different aqueous systems under direct sunlight.

MATERIALS & METHODS

Chemicals

Chlorpyrifos Technical (purity 99.9%) reference standard obtained from Sigma Aldrich and Chlorpyrifos Technical Test Item purchased from local fertilizer shop (94.56). HPLC grade acetonitrile, Methanol and GR grade formic acid, Sodium dihydrogen phosphate, Hydrochloric acid was obtained from Merck India Limited. Distilled water was purified by using the Milli-Q apparatus.

Preparation and Sterilization of Buffer Solutions

Buffer concentrations of ~0.0025 mol L-1 were used in order to minimize possible catalytic effects. The buffer solutions were labeled with pH, concentration, date of preparation, initials, and expiration date and study number. After preparation, the solutions were filtered and sterilized by passing through a 0.2-µm filter.

Sterile pH 4 Buffer

A 0.0025 mol L-1 sodium dihydrogen phosphate solution was prepared from 0.1mol L-1 sodium dihydrogen phosphate solution. This 0.0025 mol L-1 sodium dihydrogen phosphate solution was adjusted to pH 4 with 1mol L-1 hydrochloric acid. It was sterilized by passing through a 0.2 µm filter. The pH of the buffer solution was verified (4.04) following sterilization.

Instrumentation

A Shimadzu® prominence High Performance Liquid Chromatography equipped with Ultra Violet detector was used for the quantification of residues. The detector wavelength was set at 230 nm. The separation was carried out using Phenomenex® column C18 (4.6 mm i.d. and 230 mm length). The mobile phase used was Acetonitrile (82%): Water (17.5%): Acetic acid (0.5%). The flow rate was 1.0 ml per minute. The injection volume 20 µl was set for standard and sample. The peak of Chlorpyrifos was eluted at 5.0 minutes.

The residues were confirmed by analyzing the representative samples using a High Capacity Ion Trap (HCT plus) LC-MS/MS system of Bruker Daltonik GmbH. Drying gas nitrogen was generated from the pressurized air in a Nitrox UHPLCMS nitrogen generator. The nebulizer gas nitrogen flow was fixed to 10 L/min. MS/MS mode operation was done with helium as collision gas. A capillary voltage of 40 volts was used in positive ionization mode. The interface temperature was 350 °C. The scan range was 60 - 450 m/Z. Agilent 1200 HPLC system with Zorbax SB C18 column (5 µm particle size, 4.6 mm i.d., and 150 mm length), gradient elution of 0.5 ml per minute. The mobile phase used was Acetonitrile (82%): Water (17.5%): Acetic acid (0.5%). The peaks of Chlorpyrifos, O,O-diethyl hydrogen phosphorothioate, O-(amino methyl) O,O-diethyl phosphorothioate, and O-ethyl O-methyl O-(3,5,6-trichloropyridin-2-yl) phosphorothioate were eluted at 6.6 minutes, 3.5 minutes, 2.1 minutes and 0.6 minutes respectively.

Method validation

Quality criteria –Sensitivity of analytical method (Linearity of Response)

A stock solution of Chlorpyrifos standard was prepared by weighing 10.36 mg of 99.9% purity reference standard into a 25 mL volumetric flask and brought to volume with acetonitrile. A series of calibration solutions were then prepared by diluting the appropriate volume of stock solution into different 10

mL volumetric flasks and bringing to volume with acetonitrile. The prepared calibration solutions 0.02 µg/mL (CS6), 0.1 µg/mL (CS5), 0.2 µg/mL (CS4), 1.0 µg/mL (CS3), 2.0 µg/mL (CS2) and 4.0 µg/mL (CS1) were analyzed by High Pressure Liquid Chromatography using the conditions described under the Section. A linear curve was plotted for the concentration of standard versus observed peak area and the correlation coefficient was determined. A calibration curve has been presented in Figure 2.

Method Specificity

Untreated control samples of pH 4 sterile buffer solutions, acetonitrile and Test item were assayed for method specificity.

Assay accuracy and Precision

Validation includes the analysis of fortified samples and an untreated (blank) sample. Fortified samples were prepared by adding a known quantity of Chlorpyrifos reference standard solution (0.50% of solvent) to pH 4 sterile buffer solutions followed by sample work up. The quantity of Chlorpyrifos in the buffer solution was then determined by the HPLC method. The fortified samples were at 1xLOQ level = 0.06 µg/mL and 10xLOQ level = 0.6 concentration level. Method validation consisted of five replicates at each fortification level. The percentage recovery and % relative standard deviation were calculated from the response area of fortified samples.

Experimental Set-Up

The test was conducted with sterile buffer solutions of pH 4 fortified with Chlorpyrifos technical at 2 µg/mL level and taken in rectangular Quartz cell in duplicate. The test vessels were sealed. 12 test vessels were exposed to Sunlight and another 12 test vessels covered with aluminum foil were stored in a BOD incubator at 25 ± 2°C to serve as dark control. On pre-determined occasions, the samples were collected and the concentration of chlorpyrifos in the buffer was determined using the validated HPLC method.

Preparation and Application of Test Item
Preparation of Stock Solution

A stock solution of Chlorpyrifos technical (999.5 µg/mL) was prepared by weighing 102.47 mg of the test item in a 100 mL volumetric flask dissolved and brought to volume with acetonitrile. Fortification stock solution (400 ppm) was prepared by diluting the stock solution and used for further spiking.

Application of Test Item

Test solution was prepared by fortifying 0.5 mL of 400 ppm fortification stock solution in 100 mL of filter-sterilized buffer solution of pH 4 to obtain a final concentration 2µg/mL Chlorpyrifos technical. The concentration of acetonitrile used as co-solvent was 0.5 mL (less than 1%).

Sampling Procedure

The samples (both dark and irradiated) were collected at predetermined occasions, on the day 0, 3, 7 and 10 stored in the deep freezer and analyzed along with the day 15 samples. The samples of day 20 were collected, stored in deep freezer and analysed along with the day 30 samples. Before the analysis, the samples are equilibrated to room temperature and sonicated for 10 minutes.

Sample Storage Conditions

Test samples collected on sampling day were stored at <-10°C till the completion of analysis and study.

Sterility Measurements

Sterility check was determined for pH 4 buffer test systems during the study. One mL of each buffer test system was transferred into a test tube containing 9 ml of sterile distilled (10^{-1} dilution) water and thoroughly mixed using a cyclo mixer. One ml of the suspension thus obtained was further diluted into a test tube containing 9 ml of sterile distilled water (10^{-2} dilutions) and serially diluted upto 10^{-4} dilutions. One ml of suspension from 10^{-1} to 10^{-4} dilutions was taken out using a micropipette and transferred into sterilized Petri-plates aseptically using laminar air flow. Approximately, 20 ml of sterilized nutrient agar medium was uniformly distributed in to Petri-plates under laminar airflow and allowed to solidify. The plates were labeled with the inoculation date and were incubated in the BOD incubator at 35 ± 2^{o}C for two days. After incubation, all the Petri-plates were examined for determining microbial growth (Bacteria) if any compared to control Petri-plates.

Measurement of pH

The pH of test solutions was measured on day 0 and at the time of last sampling using a pH meter.

UV/ VISIBLE Absorption Measurement

The UV/VIS measurements were performed in this study using a UV-1601 UV-Visible Spectrophotometer. The path length was 1 cm, and the spectra were acquired in 1 nm increments. Test solution of 2 µg/mL Chlorpyrifos technical was prepared by diluting 0.5 mL of the fortification stock solution (400 ppm) with 100 mL of filter-sterilized buffer solution of pH 4 and used for the UV measurement. A pH 4 buffer was used as a blank.

Determination of the half-life of Chlorpyrifos technical in Tier 1: Theoretical screen Study

A single UV-Visible absorption spectrum for the test solution was measured from 400 nm to 800 nm. The test substance molar decadic absorption coefficient was calculated from 290 nm to 800 nm and the tabular solar irradiance value for summer and (preferably) 40o latitude over the same wavelength interval was noted. Maximum possible direct photolysis rate was estimated

by assuming the quantum yield in equation 1 to be equal to one and by substituting the molar decadic absorption coefficients and tabular solar irradiance values, Lλ, into equation 1. The corresponding half-life was determined.

Determination of the direct photolysis rate constant Chlorpyrifos technical in Tier 2:

Experimental study

Rectangular Quartz cells filled with the test solutions of pH 4 buffers fortified with Chlorpyrifos technical at 2 µg/mL level were taken in duplicate. The test vessels were sealed. 12 test vessels were exposed to Sunlight (13o N latitude of spring season) and another 12 test vessels covered with aluminum foil were stored in a BOD incubator at 25 ± 2°C to serve as dark control. The concentration of the test item in the irradiated test vessels and in the dark control test vessels at adequate sampling intervals was determined using a validated HPLC.

Calculations

Sample Analysis

The concentration of Chlorpyrifos technical in the samples was determined from a calibration curve using a non-linear least-squares fitting procedure. The resulting integrated equation is equivalent to:

$$Y = mx + c$$

Where,

Y = peak area of Chlorpyrifos technical in mAU*Sec

m = the slope of the line from the linear curve

x = concentration of injected sample in µg/mL

c = 'y' intercept of the linear curve

m = Slope

c = Intercept

Half-Lives

The concentrations of the test item in the irradiated test vessels and in the dark control test vessels at adequate sampling intervals were determined. The rate constant for irradiated and dark control were determined for the test item by following the equation.

$$I_{irradiated} \text{ or } d_{ark} = \frac{2.303}{t} \log_{10} \frac{C0}{Ct}$$

Where:

Ct is the concentration of test item remaining at time t, C0 is the initial concentration at time 0, and the absolute value of the slope is the first-order rate constant for the test item.

The direct photolysis rate constant

The approximate laboratory direct photolysis rate constant was computed from the following equation.

$$K_d = I_{irradiated} - d_{ark}$$

The half-life of the test item which results under summer Sunlight near the surface of a clear natural water body was estimated using these data.

$$T\ 1/2 = \frac{Ln\ (2)}{K_d} = \frac{0.693}{K_d}$$

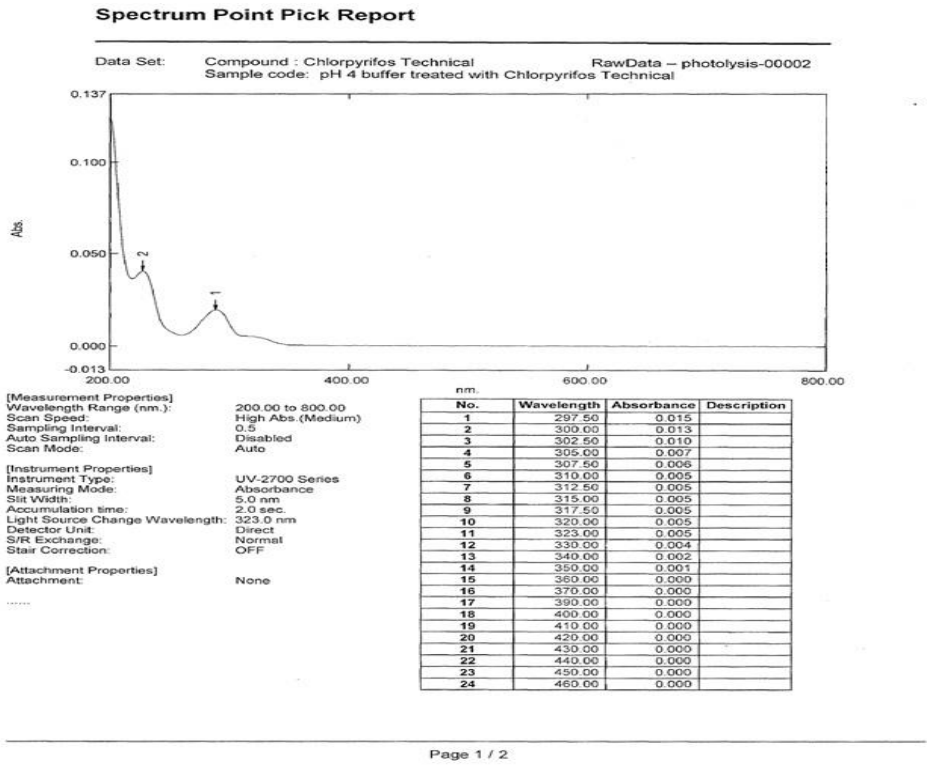
Detection Limit

The limit of quantification was 0.06 µg/mL which was less than of 10% of dose rate 2 µg/mL. HPLC analysis provided LOD as 0.02 µg/mL a sufficient signal to noise ratio to achieve a limit of detection of 1% of the injected dose.

RESULTS AND DISCUSSION

Test Item Concentration

The nominal test item concentration for this study was 1µg/mL. The actual concentration of Chlorpyrifos technical in the photolysis test vessels upon dosing was determined by HPLC method. The percentage of Chlorpyrifos technical hydrolyzed in the Tier 2 experimental serves as dark control study for pH 4 buffer solution was 1.01% at 25 ± 2°C on day 30 and the percentage of Chlorpyrifos technical photolysed in the Tier 2 -Experimental study for pH 4 buffer solution under Sunlight was 9.09% at 25 ± 2°C on day 30. The representative chromatograms are presented in Figure 9 to Figure 11



Spectrum Point Pick Report

Data Set: Compound : Chlorpyrifos Technical RawData – photolysis-00002
Sample code: pH 4 buffer treated with Chlorpyrifos Technical

[Measurement Properties]
Wavelength Range (nm.): 200.00 to 800.00
Scan Speed: High Abs.(Medium)
Sampling Interval: 0.5
Auto Sampling Interval: Disabled
Scan Mode: Auto

[Instrument Properties]
Instrument Type: UV-2700 Series
Measuring Mode: Absorbance
Slit Width: 5.0 nm
Accumulation time: 2.0 sec.
Light Source Change Wavelength: 323.0 nm
Detector Unit: Direct
S/R Exchange: Normal
Stair Correction: OFF

[Attachment Properties]
Attachment: None

No.	Wavelength	Absorbance	Description
1	297.50	0.015	
2	300.00	0.013	
3	302.50	0.010	
4	305.00	0.007	
5	307.50	0.006	
6	310.00	0.005	
7	312.50	0.005	
8	315.00	0.005	
9	317.50	0.005	
10	320.00	0.005	
11	323.00	0.005	
12	330.00	0.004	
13	340.00	0.002	
14	350.00	0.001	
15	360.00	0.000	
16	370.00	0.000	
17	390.00	0.000	
18	400.00	0.000	
19	410.00	0.000	
20	420.00	0.000	
21	430.00	0.000	
22	440.00	0.000	
23	450.00	0.000	
24	460.00	0.000	

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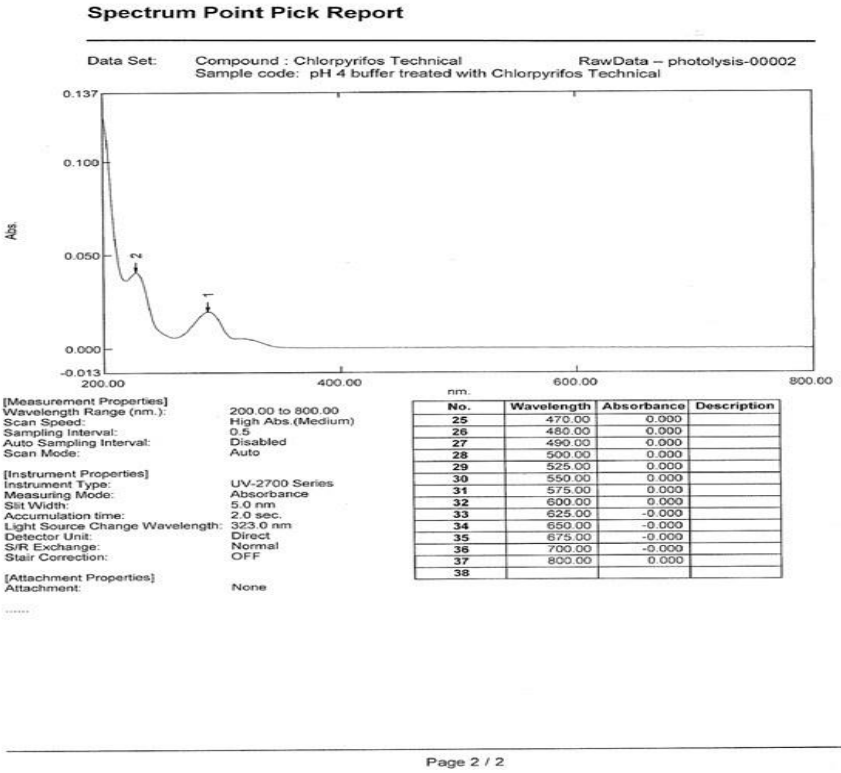
Fig 1: UV-Vis Absorption Spectra of Chlorpyrifos Technical



Spectrum Point Pick Report

Data Set: Compound : Chlorpyrifos Technical RawData – photolysis-00002
Sample code: pH 4 buffer treated with Chlorpyrifos Technical

[Measurement Properties]
Wavelength Range (nm.): 200.00 to 800.00
Scan Speed: High Abs.(Medium)
Sampling Interval: 0.5
Auto Sampling Interval: Disabled
Scan Mode: Auto

[Instrument Properties]
Instrument Type: UV-2700 Series
Measuring Mode: Absorbance
Slit Width: 5.0 nm
Accumulation time: 2.0 sec.
Light Source Change Wavelength: 323.0 nm
Detector Unit: Direct
S/R Exchange: Normal
Stair Correction: OFF

[Attachment Properties]
Attachment: None

No.	Wavelength	Absorbance	Description
25	470.00	0.000	
26	480.00	0.000	
27	490.00	0.000	
28	500.00	0.000	
29	525.00	0.000	
30	550.00	0.000	
31	575.00	0.000	
32	600.00	0.000	
33	625.00	-0.000	
34	650.00	-0.000	
35	675.00	-0.000	
36	700.00	-0.000	
37	800.00	0.000	
38			

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Fig 2: A typical HPLC calibration curve for the determination of chlorpyrifos technical

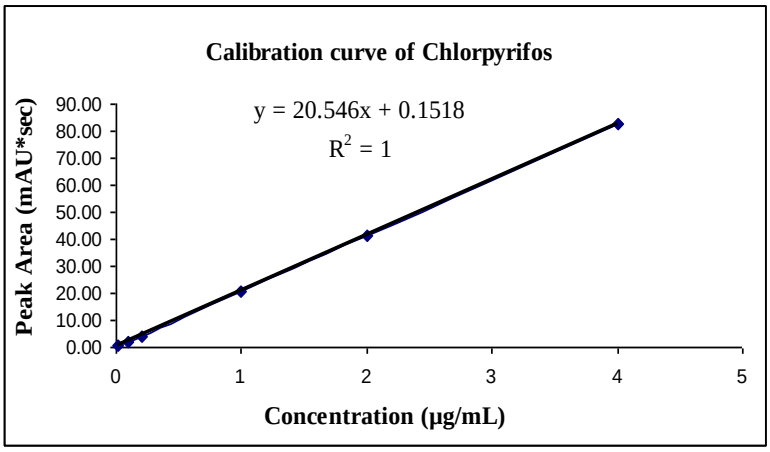


Fig 3: Linear regression analysis of the degradation data from the sterile ph 4 buffer solutions treated with chlorpyrifos - photolysis

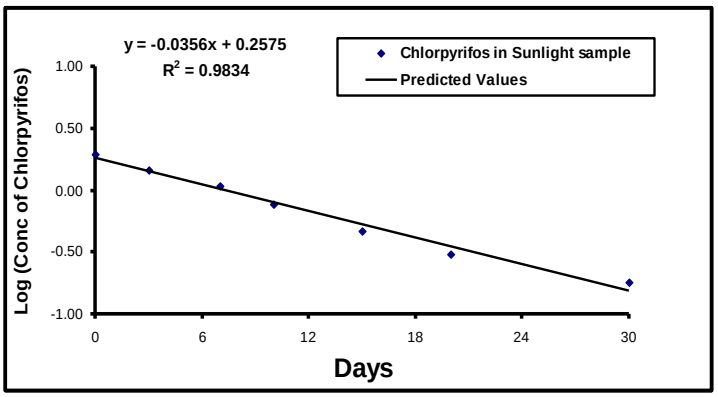


Fig 4: Linear regression analysis of the degradation data from the sterile ph 4 buffer solutions treated with chlorpyrifos technical at – dark control

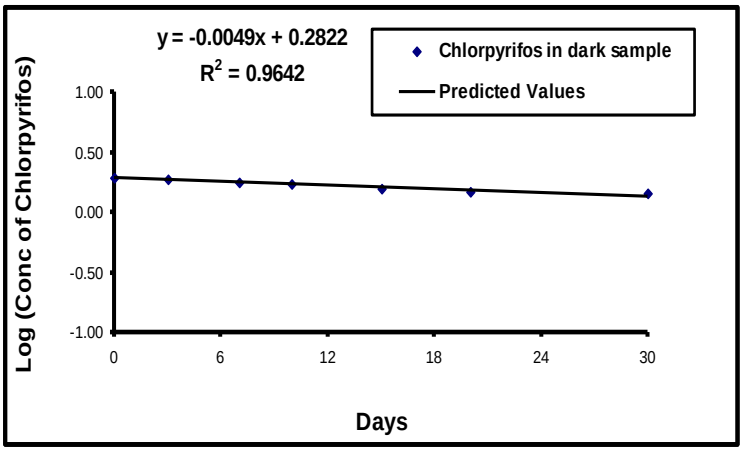


Fig 5: Representative HPLC chromatogram of recovery of chlorpyrifos standard in pH 4.0 buffer

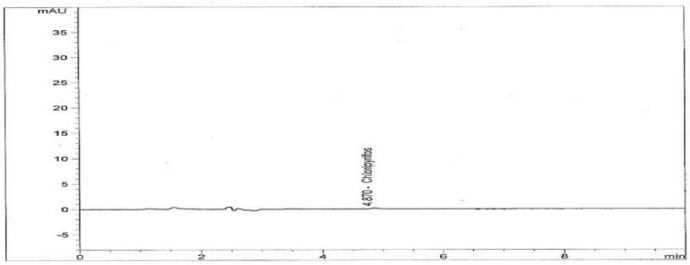


Fig 6: Representative HPLC analysis of the sterile ph 4 buffer samples treated with chlorpyrifos technical - tier 2 - experimental study – 0th day

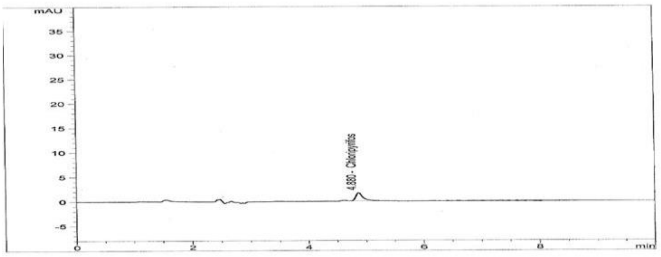


Fig 7: Representative HPLC analysis of the sterile ph 4 buffer samples treated with chlorpyrifos technical-tier 2 - experimental study – photolysis- 30th day

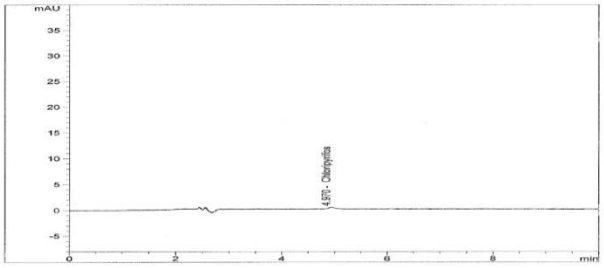


Fig 8: Representative HPLC analysis of the sterile ph 4 buffer samples treated with chlorpyrifos technical- tier 2 - experimental – dark control - 30th day

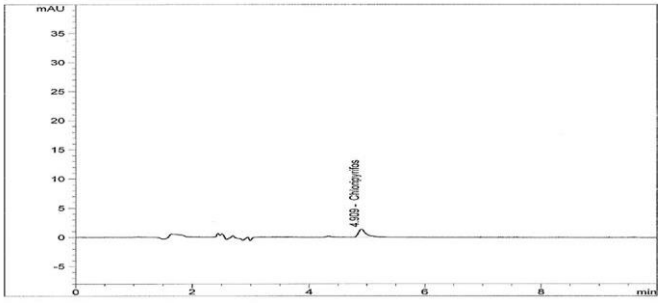
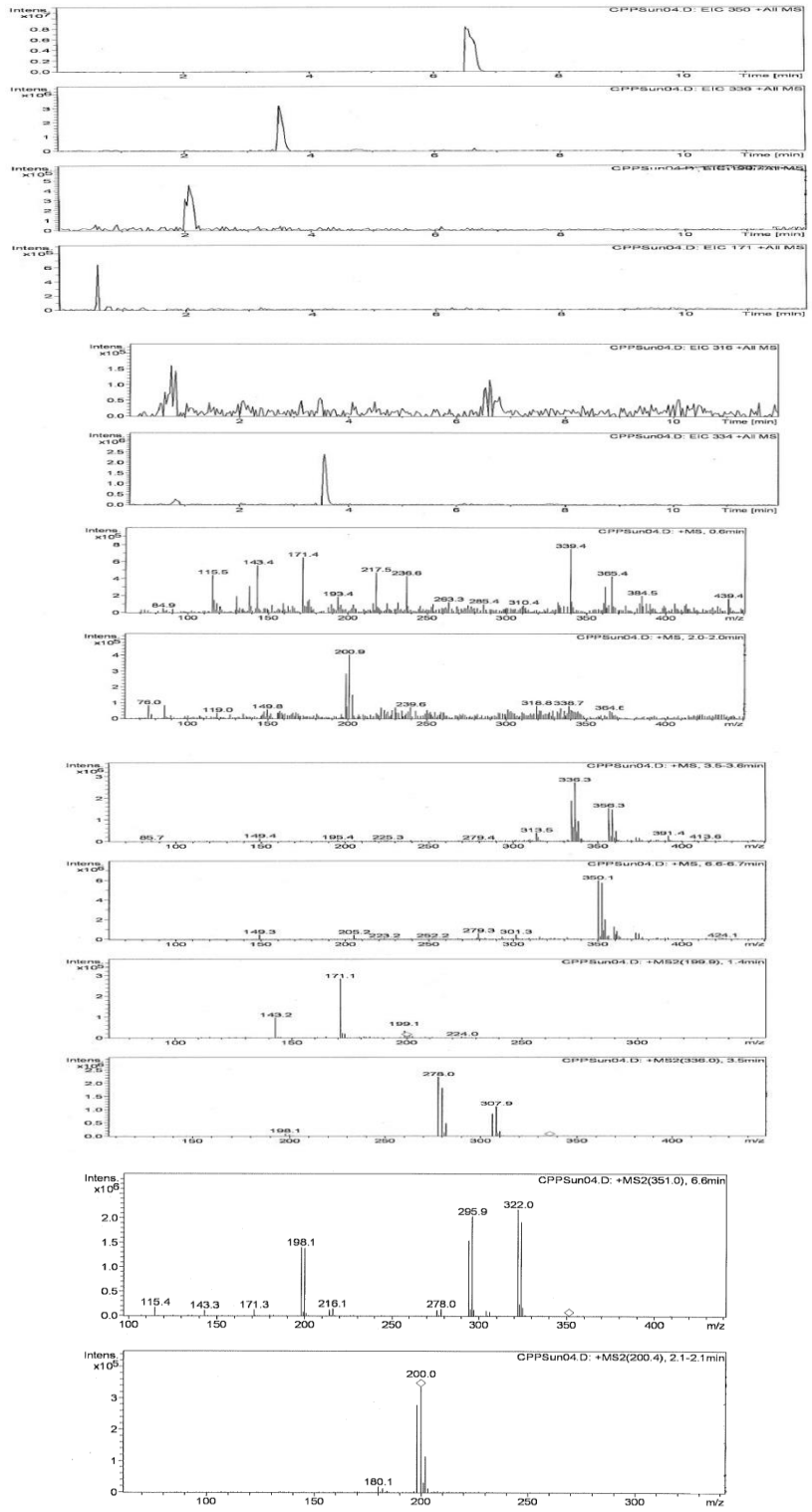


Fig 9: LC-MS Chromatogram and spectrum of Chlorpyrifos and its photoproducts in Sterile pH 4 Buffer



Verification of Sterility and pH

The sterility and pH for the photolysis test samples were measured from the Tier 2 study. The pH ranged from 4.04 to 4.02. During the sterility check, no microbial growth (bacteria) was observed in pH 4 samples at the occasions.

Validation of the Analytical Method and Validity of the Analyses

The validity of the analytical method was performed. Recoveries of the samples fortified at the 1xLOQ level = 0.06 µg/mL and 10xLOQ level = 0.6 µg/mL were determined using five samples for the validation of the method. The mean overall recovery, standard deviation and relative standard deviation (RSD) values are summarized in the table below.

Table 1: Molar decadic absorption of chlorpyrifos technical in the pH 4 buffer solution over the wave length range 290-800nm

Wave length (nm)	A_λ	C (mole L^{-1})	d (cm)	ε_λ (L $mole^{-1}$ cm^{-1})	L_λ (mmol cm^{-2} day^{-1})	$\varepsilon_\lambda.L_\lambda$ (day^{-1})
297.5	0.015	5.70E-06	1	2632	6.17E-05	1.62E-01
300.0	0.013	5.70E-06	1	2281	2.70E-04	6.16E-01
302.5	0.010	5.70E-06	1	1754	8.30E-04	1.46E+00
305.0	0.007	5.70E-06	1	1228	1.95E-03	2.39E+00
307.5	0.006	5.70E-06	1	1053	3.74E-03	3.94E+00
310.0	0.005	5.70E-06	1	877	6.17E-03	5.41E+00
312.5	0.005	5.70E-06	1	877	9.07E-03	7.96E+00
315.0	0.005	5.70E-06	1	877	1.22E-02	1.07E+01
317.5	0.005	5.70E-06	1	877	1.55E-02	1.36E+01
320.0	0.005	5.70E-06	1	877	1.87E-02	1.64E+01
323.0	0.005	5.70E-06	1	877	3.35E-02	2.94E+01
330.0	0.004	5.70E-06	1	702	1.16E-01	8.14E+01
340.0	0.002	5.70E-06	1	351	1.46E-01	5.12E+01
350.0	0.001	5.70E-06	1	175	1.62E-01	2.84E+01
360.0	0.000	5.70E-06	1	0	1.79E-01	0.00E+00
370.0	0.000	5.70E-06	1	0	1.91E-01	0.00E+00
380.0	0.000	5.70E-06	1	0	2.04E-01	0.00E+00
390.0	0.000	5.70E-06	1	0	1.93E-01	0.00E+00
400.0	0.000	5.70E-06	1	0	2.76E-01	0.00E+00
410.0	0.000	5.70E-06	1	0	3.64E-01	0.00E+00
420.0	0.000	5.70E-06	1	0	3.74E-01	0.00E+00
430.0	0.000	5.70E-06	1	0	3.61E-01	0.00E+00
440.0	0.000	5.70E-06	1	0	4.26E-01	0.00E+00
450.0	0.000	5.70E-06	1	0	4.80E-01	0.00E+00
460.0	0.000	5.70E-06	1	0	4.85E-01	0.00E+00
470.0	0.000	5.70E-06	1	0	5.02E-01	0.00E+00
480.0	0.000	5.70E-06	1	0	5.14E-01	0.00E+00
490.0	0.000	5.70E-06	1	0	4.86E-01	0.00E+00
500.0	0.000	5.70E-06	1	0	4.96E-01	0.00E+00
525.0	0.000	5.70E-06	1	0	1.31E+00	0.00E+00
550.0	0.000	5.70E-06	1	0	1.36E+00	0.00E+00
575.0	0.000	5.70E-06	1	0	1.37E+00	0.00E+00
600.0	0.000	5.70E-06	1	0	1.38E+00	0.00E+00
625.0	0.000	5.70E-06	1	0	1.40E+00	0.00E+00
650.0	0.000	5.70E-06	1	0	1.41E+00	0.00E+00
675.0	0.000	5.70E-06	1	0	1.41E+00	0.00E+00
700.0	0.000	5.70E-06	1	0	1.40E+00	0.00E+00
750.0	0.000	5.70E-06	1	0	2.69E+00	0.00E+00
800.0	0.000	5.70E-06	1	0	2.59E+00	0.00E+00
					$k_{d(solar)}$	253.07
					$t_{1/2}$ (day)	2.74E-03

Table 2: The %recovery data for these samples

Percentage of Recovery	Level	
	1 x LOQ	Higher Level
Mean	96.19	97.57
Standard Deviation	1.68	0.75
%RSD	1.75	0.77

Table 3: percent recovery of the method validation in pH 4 buffer treated with chlorpyrifos standard

Sample code	Recovery Percentage In pH 4 Buffer	Mean % Recovery	Standard deviation	% Relative Standard deviation
1xLOQ-R1	94.89	96.19	1.68	1.75
1xLOQ-R2	96.51			
1xLOQ-R3	98.13			
1xLOQ-R4	97.32			
1xLOQ-R5	94.08			
10xLOQ-R1	97.24	97.57	0.75	0.77
10xLOQ-R2	98.38			
10xLOQ-R3	96.84			
10xLOQ-R4	98.38			
10xLOQ-R5	97.00			

Table 4: photolysis under direct sun light- concentration of chlorpyrifos technical in the pH 4 buffer solution

Photolysis Under Sunlight							
Sampling occasions	**Day 0**	**Day 3**	**Day 7**	**Day 10**	**Day 15**	**Day 20**	**Day 30**
Replication 1 (µg/mL)	1.95	1.46	1.09	0.79	0.48	0.33	0.19
Replication 2 (µg/mL)	1.95	1.43	1.06	0.76	0.46	0.28	0.17
Mean (µg/mL)	0.29	0.16	0.03	-0.12	-0.34	-0.55	-0.77
Mean (log µg/mL)	1.95	1.45	1.08	0.78	0.47	0.31	0.18
Dark control							
Sampling occasions	**Day 0**	**Day 3**	**Day 7**	**Day 10**	**Day 15**	**Day 20**	**Day 30**
Replication 1 (µg/mL)	1.95	1.87	1.76	1.67	1.58	1.50	1.43
Replication 2 (µg/mL)	1.95	1.86	1.78	1.70	1.56	1.48	1.37
Mean (µg/mL)	0.29	0.27	0.25	0.23	0.19	0.17	0.14
Mean (log µg/mL)	1.95	1.87	1.77	1.69	1.57	1.49	1.40

Table 5: Kinetic analysis of chlorpyrifos technical in the pH 4 buffer solution

Matrix	Rate constant (day^{-1})	Half-life (day)	r^2
Photolysis under Sunlight (Total)	$k_{irradiated}$ - 0.08189	8.46	0.9834
Dark control	k_{dark} - 0.01126	61.55	0.9642
Direct photolysis Under Sun light- (Actual)	k_d - 0.07119	9.73	-

The recovery data for these samples are summarized in Table 3. The representative chromatograms are presented in Figure 8.

Determination of the half-life of Chlorpyrifos technical in the Tier 1: Theoretical screen Study

Tier 1: Theoretical screen of photolysis test for Chlorpyrifos technical was conducted to determine the approximate half life. The approximate half life of Chlorpyrifos Technical was 2.74 x 10-3 day. The data are presented in Table 1. Based on the data generated in this study, Chlorpyrifos technical was found to be photolytically degradable in pH 4.

Determination of the Concentration of Chlorpyrifos technical in the Tier 2 -Experimental Study

Based on the Tier 1: Theoretical screen study, Tier 2: An Experimental study was conducted for test solution under direct Sunlight at 25 ± 2°C. The concentration of Chlorpyrifos technical in the test

samples was determined using a calibration curve generated with each set of analyses.

The concentration of Chlorpyrifos technical in the test solution exposed to Sunlight at 25 ± 2°C was 1.95 µg/mL on the 0 day and 0.18 µg/mL on the 30th day. The concentration of Chlorpyrifos technical in the test solution stored at 25 ± 2°C in the dark was 1.95 µg/mL on 0 day and 1.40 µg/mL on the 30th day. The above results are presented in Table 4.

LC-MS analysis was done to identify the degradation product of Chlorpyrifos Technical in both, irradiated and non-irradiated samples on day 0, 3, 7, 10, 15, 20, and 30. The degradation products identified in the irradiated samples are O, O-diethyl hydrogen phosphorothioate, O-(amino methyl) O, O-diethyl phosphorothioate, and O-ethyl O-methyl O-(3, 5, 6-trichloropyridin-2-yl) phosphorothioate on day 3, day 7, and day 10 in Chlorpyrifos Technical. No degradation product was identified in the dark control Chlorpyrifos Technical samples. The representative Mass spectrums are presented in Figure 12.

Kinetic Analysis of Data

Degradation kinetics of Chlorpyrifos technical was determined in a sterile buffer solution of pH 4. Table 4 contains the amount of Chlorpyrifos technical at each sampling time of photolysis and dark control. Degradation kinetics for the parent chlorpyrifos technical in pH 4 sterile buffer solution has been presented in Figure 3 and Figure 4. A summary of the kinetic analysis has been provided in Table 5.

Chlorpyrifos technical was determined to be undergoing aqueous abiotic photolysis at pH 4 and is expected to contribute to the degradation of Chlorpyrifos technical significantly.

Experiment	**Rate constant (day^{-1})**	**Half life (days)**
Photolysis	$i_{irradiated}$ - 0.08189	8.46
Dark control	d_{ark} - 0.01126	61.55
Direct photolysis	K_d - 0.07119	9.73

CONCLUSION

On the basis of these results, aqueous abiotic photolysis would not be expected to contribute significantly to the degradation of Chlorpyrifos technical at pH 4.

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REFERENCES

1. Phillip W Graebing, J S Chib, Terrance D Hubert, William H Gingerich; Aqueous photolysis of niclosamide. J Agric Food Chem, 2004; 52(4): 870-878.
2. Filippo M. Pirisi, Paolo Cabras, Luigi Garau V, Marinella Melis, Eurico Secchi; Photo degradation of Pesticides. Photolysis Rates and Half-Life of Pirimicarb and its metabolites in reactions in Water and in Solid Phase, Agric. Food Chem, 1996; 44(8): 2417-2422.
3. Filippo M. Pirisi, Alberto Angioni, Maddalene Cabizza, Paolo Cabras, Clara Falqui Cao; Photolysis of pesticides: Influence of epicuticular waxes from Persica laevis DC on the photo degradation in te solid phase of aminocarb, methiocarb and fenthion, 2001; 57(6): 522-526.
4. Wang L, Li Q.X, Cai W.F; Photolysis of phloxine B in water and aqueous solutions, Environmental Contamination & Toxicology, 1998; 35(3): 397.
5. Pirisi FM, Cabras P, Garau VL, Melis M, Secchi E; Photo degradation of pesticides, photolysis rates and half-life of pirimicarb and its metabolites in reactions in water and in soil phase, Journal of Agricultural and Food Chemistry, 1996; 44(8): 2417-2422.
6. Graebing P1, Chib JS; Soil photolysis in a moisture- and temperature-controlled environment. 2. Insecticides. J Agric Food Chem, 2004; 52(9):2606-2614.
7. Theodore A. Slotkin, Frederic J. Seidler, Changlong Wu, Emiko A. MacKillop, Karl G. Linden; Ultraviolet Photolysis of Chlorpyrifos Developmental Neurotoxicity Modeled in PC12 Cells. Environ Health Perspect, 2009; 117(3): 338–343.
8. Meikle RW, Kurihara NH, DeVries DH, Chlorpyrifos; The photodecomposition rates in dilute aqueous solution and on a surface, and the volatilization rate from a surface. Archives of Environmental Contamination and Toxicology, 1983; 12(2): 189-193.
9. Phillip Graebing, Chib JS; Soil Photolysis in a Moisture- and Temperature-Controlled Environment. 2. Insecticides. J. Agric. Food Chem, 2004; 52 (9): 2606–2614.
10. Ekrem Karpuzcua M, David L. Sedlaka C, William T. Stringfellowb; Biotransformation of chlorpyrifos in riparian wetlands in agricultural watersheds: Implications for wetland management. Journal of Hazardous Materials, 2013; 244:111– 120.

11. Augustine Chioma Affam, Malay Chaudhuri; Degradation of pesticides chlorpyrifos, cypermethrin and chlorothalonil in aqueous solution by TiO2 photo catalysis. Journal of Environmental Management, 2013; 130: 160–165.
12. Mbugua JK, Mbui DN, Kamau GN; Adsorption of Dursban (Chlorpyrifos) Pesticide by Loam Soil from Limuru, Kenya: Apparent Thermodynamic Properties. Africa Journal of Physical Sciences, 2014; (1): 1-9.
13. Xiao Zhang, Yan Shen, Xiang-yang, Yu Xian-jin Liu; Dissipation of chlorpyrifos and residue analysis in rice, soil and water under paddy field conditions. Ecotoxicology and Environmental Safety, 2012; 78: 276–280.