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LABORATORY STUDY OF HYDROLYSIS OF TEMEPHOS TECHNICAL IN DIFFERENT STERILE BUFFER SOLUTIONS BY VALIDATED HPLC-DAD METHOD

Chemistry

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ABSTRACT

A laboratory study was conducted to investigate the hydrolysis of temephos technical, an organophosphate insecticide, in different buffer solutions using validated high-performance liquid chromatography with diode array detection (HPLC-DAD) method. The buffer solutions were prepared at pH values of 4, 7 and 9. The hydrolysis reactions were carried out at 25°C and 35°C for 30 days. The HPLC-DAD method was validated for linearity, accuracy, precision, limit of detection, limit of quantification and robustness, and showed satisfactory results for all the parameters. The hydrolysis of temephos followed first-order kinetics in all the buffer solutions, and the rate constants and half-lives were calculated. The results of this study at 50 ± 0.5°C showed that Temephos Technical was not hydrolytically stable at pH 4, pH 7 and pH 9 buffer solutions. The table below shows the percent degradation at each pH after 5 days of incubation hydrolysis rate increased with increasing pH, indicating that temephos was more stable in acidic conditions than in alkaline conditions. The results of this study provide useful information for the environmental fate and risk assessment of temephos in aquatic ecosystems.

KEYWORDS

Temephos Technical, Hydrolysis, Buffer Solutions, Hplc-dad, First-order Kinetics

INTRODUCTION

Temephos is an organophosphate insecticide that belongs to the class of phosphorothioates. It is used as a larvicide to control the population of mosquitoes and other insects that can transmit diseases to humans, such as midges, black flies and cyclops [1]. Temephos acts by inhibiting the enzyme cholinesterase, which is essential for the proper functioning of the nervous system [2]. Temephos is applied to water sources where the larvae of these insect's breed, such as ponds, marshes, swamps and containers. Temephos is insoluble in water and has a low vapor pressure, which means it does not evaporate easily [3]. Temephos is relatively stable in the environment, but it can be degraded by sunlight, microorganisms and hydrolysis [4]. Temephos has a low acute toxicity to mammals, birds and fish, but it can cause adverse effects if ingested or inhaled in large amounts [5]. Temephos can also affect non-target organisms, such as bees and aquatic invertebrates. Therefore, temephos should be used with caution and according to the label instructions [6].

Hydrolysis is a chemical reaction in which water molecules are split into hydrogen and hydroxide ions. Hydrolysis can occur in various types of compounds, such as salts, acids, bases, esters, and amides [7]. In this laboratory study, we investigated the factors that affect the rate and extent of hydrolysis of different substances [8]. We used a pH meter to measure the change in acidity or alkalinity of the solutions over time [9]. We also used indicators, such as phenolphthalein and bromothymol blue, to observe the color changes that indicate the presence of hydroxide or hydrogen ions. We varied the temperature, concentration, and nature of the substances to see how they influenced the hydrolysis process [10]. We found that hydrolysis is faster and more complete at higher temperatures, higher concentrations, and with substances that have weaker bonds or higher polarity. We also learned how to calculate the equilibrium constant and the degree of hydrolysis for each reaction [11]. Our results demonstrate the importance of hydrolysis in many chemical and biological processes, such as digestion, metabolism, and corrosion.

Pesticides are widely used in agriculture to control pests and diseases, but they may also pose risks to human health and the environment. One of the factors that affects the fate and transport of pesticides in the environment is hydrolysis, which is the chemical reaction of a pesticide with water. Hydrolysis can result in the degradation or transformation of the pesticide into less toxic or more toxic compounds, depending on the type and structure of the pesticide. Hydrolysis can also affect the bioavailability and persistence of pesticides in soil and water [12].

The rate and extent of hydrolysis depend on several factors, such as the pH, temperature, and presence of catalysts or inhibitors in the aqueous medium. Therefore, it is important to study the hydrolysis behavior of pesticides under different environmental conditions to assess their potential impact and risk. In this paper, we review the methods and techniques used to measure and model the hydrolysis of pesticides, as well as the main findings and challenges in this field of research. We also discuss the implications of hydrolysis for the environmental fate and risk assessment of pesticides [13].

Hydrolysis is a chemical reaction that involves the breaking of bonds between molecules by the addition of water. Hydrolysis can affect the stability, solubility, and bioavailability of drugs and other substances [14]. High-performance liquid chromatography (HPLC) is a technique that can separate and quantify the components of a mixture based on their physical and chemical properties. HPLC can be used to study the hydrolysis of various compounds under different conditions, such as pH, temperature, and catalysts.

In this laboratory study, we will use HPLC to investigate the hydrolysis of aspirin, a common analgesic and anti-inflammatory drug, in acidic and alkaline solutions. We will also compare the results with those obtained from a spectrophotometric method, which measures the absorbance of light by a solution at a specific wavelength. The objectives of this study are to understand the principles and applications of HPLC and hydrolysis, to develop the skills and techniques for performing HPLC analysis, and to evaluate the accuracy and precision of the methods used.

In this study, we developed and validated a high-performance liquid chromatography with diode array detection (HPLC-DAD) method to measure the concentration of temephos in different buffer solutions. We investigated the effects of pH, temperature, and buffer type on the hydrolysis kinetics of temephos technical. This study provides useful information for the application and environmental fate of temephos in different aquatic systems.

Expermental

Preparation of solutions

Preparation of Mobile Phase-D: (0.1% Ortho phosphoric acid)

Accurately pipetted out 1.000 mL of Orthophosphoric acid in to 1000 mL of mobile phase bottle containing 500 mL of HPLC Grade Water. Mixed well by shaking and sonicated to degassed. Made up to mark with same Water. The mobile phase 1000 mL was prepared once per week until the end of the experiment.

Preparation of Mobile Phase-B: (Acetonitrile)

Acetonitrile Sonicated to degassed.

Preparation of Diluent

Pure HPLC grade Acetonitrile was used for diluent.

Preparation and Sterilization of Buffer Solutions
Preparation 0.1M Hydrochloric acid solution
Accurately 8.4 mL of concentrated hydrochloric acid solution was pipetted out into a 1000 mL volumetric flask containing 250 mL of water. The volume of the solution was made up to the mark using water. The solution was sonicated for 15 minutes to mix the content.

Preparation 0.1M Mono potassium phosphate solution
Accurately 6.82 g of potassium dihydrogen ortho phosphate was weighed and transferred into a 500 mL volumetric flask. About, 200 mL of water was added to it and the solution was sonicated for 15 minutes to mix the content. The volume of the solution will be made up to the mark using water and mixed well.

Preparation 0.1M Mono potassium citrate solution
Accurately 16.21 g of mono potassium citrate was weighed and transferred into a 500 mL volumetric flask. About, 500 mL of water was added to it and the solution was sonicated for 15 minutes to mix the content. The volume of the solution was made up to the mark using water and mixed well.

Preparation 0.1M Sodium hydroxide solution
Accurately 2.11 g of sodium hydroxide was weighed and transferred into a 500 mL volumetric flask. About, 200 mL of water was added slowly to it and the solution was sonicated for 15 minutes to mix the content. The volume of the solution was made up to the mark using water and mixed well.

Preparation 0.1M Boric acid (H_3BO_3) in 0.1M Potassium chloride (KCl) solution
Accurately 3.01 g of boric acid and 3.73 g of potassium chloride was weighed and transferred into a 500 mL volumetric flask. About, 500 mL of water was added slowly to it and the solution was sonicated for 15 minutes to mix the content. The volume of the solution was made up to the mark using water and mixed well.

Preparation Sterile acidic buffer solution (pH = 4.0)
Accurately 45 mL of 0.1M sodium hydroxide solution and 250 mL of 0.1M potassium citrate solution was pipetted out into a 250 mL volumetric flask and the volume of the solution was made up to the mark using water. The solution was shaken well. The pH of the solution was adjusted by using 0.1M hydrochloric acid solution and mixed well. The pH of the buffer solution was verified (4.01) and filtered through 0.45 µm membrane filter.

Preparation Sterile neutral buffer solution (pH = 7.0)
About 74 mL of 0.1M sodium hydroxide solution and 125 mL of 0.1M mono potassium phosphate solution was pipetted out into a 250 mL volumetric flask and the volume of the solution was made up to the mark using water. The solution was shaken well. The pH of the solution was adjusted by using 0.1M sodium hydroxide and 0.1M hydrochloric acid solution and mixed well. The pH of the buffer solution was verified (7.02) and filtered through 0.45 µm membrane filter.

Preparation Sterile basic buffer solution (pH = 9.0)
About 53.3 mL of 0.1M sodium hydroxide solution and 125 mL of 0.1M boric acid in 0.1M KCl solution was pipetted out into a 250 mL volumetric flask and the volume of the solution was made up to the mark using water. The solution was shaken well. The pH of the solution was adjusted by using 0.1M sodium hydroxide and 0.1M hydrochloric acid solution and mixed well. The pH of the buffer solution was verified (9.01) and filtered through 0.45 µm membrane filter.

Experimental Apparatus
Individual test systems were composed of sterile buffer containing the test material in separate amber glass bottles with screw caps. For the preliminary test, the individual hydrolysis test vessels were placed in a Hot air oven set at 50 ± 0.5°C. For the definitive test at pH 9 the individual hydrolysis test vessels were placed in a BOD incubator set at 20 ± 0.5°C and 35 ± 0.5°C.

Description of Experimental Set-up
The test was conducted with sterile buffer solutions of pH 4, 7 and 9 fortified with Permethrin technical at 1 µg/mL level and taken in glass bottles in duplicate. The test vials were capped, wrapped with aluminum foil and stored in BOD incubator in dark. On pre-determined occasions, the samples were processed and the concentration in the buffer was determined using validated HPLC method.

Temperature Control
The sample temperature for preliminary test was maintained at 50 ± 0.5°C throughout the duration of the study and it was continuously monitored during test.

The sample temperature for definitive test was maintained at 20 ± 0.5°C and 35 ± 0.5°C during study and it was continuously monitored.

Preparation of Test item Stock Solution
A stock solution of Temephos (1000.24 mg/L) was prepared by weighing 10.43 mg of test item in 10 ml volumetric flask and brought to volume with methanol and used for further spiking.

Application of Test Item
Test solution was prepared by fortifying the stock solution in filter-sterilized buffer solution to obtain a final concentration 1 mg/L Temephos. The concentration of methanol used as co-solvent was 0.7 mL (less than 1%).

For the preliminary and definitive test, the test solutions were prepared in a sterile amber coloured glass bottles and covered with aluminium foil. The test vessels were labeled for pre-decided sampling occasions.

Test System Maintenance and Sampling
A summary of the test system maintenance, sampling intervals and sampling procedures are listed in Table 3 (Preliminary study) and Table 4 (Definitive study).

Sterility Measurements
Sterility check was determined for pH 4, 7 and 9 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 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 micropipette and transferred into sterilized petriplates 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 inoculation date and were incubated in BOD incubator at 35 ± 2oC for two days. After incubation, all the petriplates was examined for determining microbial growth (Bacteria) if any compared to control petriplates.

Chromatographic Conditions
The analysis was performed using a equipped with Agilent HPLC 1260 Series and DAD detector. The column used was a Phenomenex C18 (150 mm length x 4.6mm i.d x 5 µm film thickness).The mobile phase consisted of a mixture of acetonitrile (85%) and 0.1% OPA (15%).The column temperature was maintained at 30°C. The flow rate was 1.0 ml/min. The run time was set to 12 minutes. The retention time of the analyte was approximately 5.4 min.

Method Validation
Specificity Test
- Following solutions were injected in specificity test
- Diluent Blank (Acetonitrile)
- Mobile Phase-D (0.1 % Orthophosphoric acid solution in water)
- Mobile phase-B (Acetonitrile)
- Prepared pH-4, pH-7 and pH-9 buffer controls
- Reference Item Solution in Prepared pH-4, pH-7 and pH-9 buffers (Accuracy Solution-
- LOQ Level)
- Reference Item Solution (1.00 mg/L-Linearity-3 solution)
- 1.0 mg/L Test Item Solution

Linearity Test
Accurately 10.02 mg of reference item (99.81%) was weighed into 10 mL volumetric flask. Contents were dissolved in about 5 mL of methanol and make upto mark with the same. The stock solution concentration was 1000 mg/L. The linearity stock solutions were diluted in following way.

Standard Stock Solution Concentration (µg/mL)	Aliquot of stock taken (mL)	Made up to Volume (mL)	Concentration of solution prepared (µg/mL)	Solution ID
1000	1.000	10	100.00	Linearity-6
1000	0.500	10	50.00	Linearity-5
1000	0.100	10	10.00	Linearity-4
10	1.000	10	1.00	Linearity-3
1.0	0.500	10	0.05	Linearity-2
1.0	0.150	10	0.015	Linearity-1

All the linearity solutions were analyzed by High Pressure Liquid Chromatography using the conditions described conditions. A linear curve was plotted for the concentration of standard versus observed peak area and the correlation coefficient was determined.

Recovery and Repeatability
Accuracy was performed at two fortification levels (0.05 µg/mL and 0.5 µg/mL).

In pH-4, pH-7 and pH-9 Buffer Solutions
0.05 mg/L fortification level
Accurately pipetted out 0.500 mL of 1.00 mg/L reference item solution was taken into fifteen different 10 mL of volumetric flasks and made to the mark with three buffers, then sonicated for 5 minutes for homogenization. Samples were prepared in five replicates LOQ Level (R1, …R5).

0.5 mg/L fortification level
Accurately pipetted out 0.500 mL of 10.00 mg/L specificity test item solution was taken into fifteen different 10 mL of volumetric flasks and made to the mark with three buffers, then sonicated for 5 minutes for homogenization. Samples were prepared in five replicates (R1, …..R5).

Limit of Quantification
The lowest validated level with sufficient recovery is defined as the limit of quantification (LOQ). The LOQ was determined using 5 injections of 0.05 mg/L fortification level recovery samples. Low level recovery solutions were used for LOQ.

Limit of Detection
The limit of detection (LOD) is defined as the lowest detectable concentration of an analytic in a sample. It was expressed as lowest calibration Standard in linearity test. Limit of detection was established by dividing the concentration of the LOQ with 3.3 values.

Calculations
Treatment Rate
The following example calculation was used in determining the amount of stock solution of Temephos (Concentration = 100 mg/L) for the nominal treatment level of 1 mg/L in 100 mL of sterile buffer dosing solution.

Total volume of sample = 100 mL
100 mL x 1 mg/L = X mL x 100 mg/L
X mL = 1.000 mL

Half-Lives
The degradation rate expressed as the concentration (µg/mL) in the buffer solution as Permethrin technical was estimated from fitting the data to the following equation.

$$Kobs = \frac{2.303}{t} \log_{10} \frac{C0}{Ct}$$

$$T_{1/2} = 0.693 / Kobs$$

Where, Kobs is the rate constant.

A linear regression analysis of log10 (C) versus time derives an equation whose slope = -k. The half-life (t1/2) is therefore calculated as 0.693/Kobs.

RESULTS AND DISCUSSION
Specificity
The analytical method was found to be specific. There was no interference observed at the retention time of the Temephos peak.

Linearity
The analytical method was found to be linear in the range 0.015 mg/L to 100.0 mg/L with Correlation co-efficient (r) =0.9999, Slope = 32.81 and Intercept = 0.39. The results of area of concentrations were presented in **Table 1**. The linearity curve presented in **Figure 1.**

Table 1 Detector Linearity Test

Concentration in mg/L	Peak Area
0.015	0.301
0.050	1.526
1.00	32.801
10.00	309.256
50.00	1678.287
100.00	3264.231
Slope	32.81
Intercept	0.39
CC	0.9999

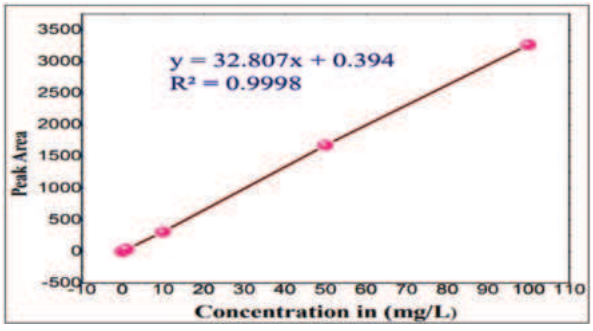

Figure 1: Calibration Curve of Temephos Standard

Recovery and Repeatability
Multiple recovery control samples (n=2) at each of 2 fortification levels equivalent to 0.05 mg/L and 0.5 mg/L for both buffers and octanol phase. Temephos technical in the final solution were assayed using HPLC.

For RO water, the overall assay accuracy of 88.92% recovery and a precision of 1.41 % RSD indicated an acceptable method of analysis at LOQ Level. The overall assay accuracy of 97.57% recovery and precision RSD of 0.97% indicated an acceptable method of analysis in high concentration.

For pH-4 buffer, the overall assay accuracy of 92.92 % recovery & precision RSD of 1.31% at LOQ level and 88.70 % recovery & precision RSD of 0.87 % at LOQ x 10 level. For pH-4 buffer, the overall assay accuracy of 85.48 % recovery & precision RSD of 2.29% at LOQ level and 86.08 % recovery & precision RSD of 1.78 % at LOQ x 10 level. For pH-9 buffer, the overall assay accuracy of 85.80 % recovery & precision RSD of 2.20 % at LOQ level and 84.72 % recovery & precision RSD of 0.80 % at LOQ x 10 level.

Limit of Quantitation (LOQ)
Limit of Quantitation (LOQ) was established to be 0.050 mg/L from the lower level recovery test in RO water and n-Octanol.

Limit of Detection (LOD)
Limit of Detection (LOD) was established to be 0.015 mg/L and that was lowest limit of calibration curve.

Verification of Sterility and pH
Measured the sterility and pH for the hydrolysis test samples from the definitive on pH 4, 7 and 9 samples. The pH ranged from 4.01 to 4.05, 7.02 to 7.06 and 9.02 to 9.04. During the sterility check, no microbial growth (bacteria) was observed in pH 4, 7 and 9 samples at the occasions.

Determination of the Concentration of Temephos in the Preliminary Study
The preliminary hydrolysis test for Temephos Technical was conducted at 50 ± 0.5°C in the dark at each of pH 4, 7 and 9 for 5 days. The percent degradation at each pH after 5 days of incubation were presented below.

68% hydrolysis was observed in pH 4 buffer.

59% hydrolysis was observed in pH 7 buffer
72% hydrolysis was observed in pH 9 buffer.
Based on the data generated in this study, Temephos Technical was hydrolytically unstable in pH 4, pH 7 and pH 9 at 50°C.

Determination of the Concentration of Temephos in the Definitive Study at pH 9

Based on the preliminary test the definitive studies were conducted in pH 4, pH 7 and pH 9 at 20 ± 0.5°C and 35 ± 0.5°C. The concentration of Temephos in the test samples was determined using a standard area method.

The concentration of Temephos in the sterile buffer solution of pH 4 incubated at 20°C was 1.006 mg/L on 0 day and 0.125 mg/L on 30th day. The concentration of Temephos in the sterile buffer solution of pH 4 incubated at 35°C was 1.002 mg/L on 0 day and BDL on 30th day.

The concentration of Temephos in the sterile buffer solution of pH 7 incubated at 20°C was 1.001mg/L on 0 day and 0.102 mg/L on 30th day.

The concentration of Temephos in the sterile buffer solution of pH 7 incubated at 35°C was 1.003 mg/L on 0 day and BDL on 30th day.

The concentration of Temephos in the sterile buffer solution of pH 9 incubated at 20°C was 1.004mg/L on 0 day and 0.06 mg/L on 30th day.

The concentration of Temephos in the sterile buffer solution of pH 9 incubated at 35°C was 1.002 mg/L on 0 day and BDL on 30th day.

Kinetic Analysis of Data

Degradation kinetics of Temephos was determined in sterile buffer solution of pH 4, 7 and 9. The details are presented in **Table.2, Table 3** contains amount of Temephos at each sampling time. Degradation kinetics for the parent Temephos in pH 4, 7 and 9 sterile buffer solutions is presented figures presented in **Figure 2and figure 3.**

Table 2 Kinetic Analysis of Temephos in the pH 4 Buffer Solution

Rate Constant and t1/2 - pH 4.0 at 20° C			Rate Constant and t1/2 - pH 4.0 at 35° C		
Sampling occasion (Days)	Log of concentration	Rate Constant	Sampling occasion (Days)	Log of concentration	Rate Constant
0.04	0.0014	-	0.04	0.015	-
1	-0.074	0.1809	1	-0.095	0.2639
5	-0.085	0.0063	5	-0.672	0.3322
10	-0.148	0.0290	10	-0.769	0.0447
15	-0.127	-0.0097	15	-0.895	0.0580
20	-1.357	0.5665	20	NC	NC
30	-1.742	0.0887	Average Rate Constant		0.1747
Average Rate Constant		0.1436	t1/2 (days)		3.97
t1/2 (days)		4.83	CC		-0.9185
CC		-0.8955			

Table 3 Kinetic Analysis of Temephos in the pH 7 Buffer Solution

Rate Constant and t1/2 - pH 7.0 at 20° C			Rate Constant and t1/2 - pH 7.0 at 35° C		
Sampling occasion (Days)	Log of concentration	Rate constant	Sampling occasion (Days)	Log of concentration	Rate constant
0.04	0.021	-	0.04	0.018	-
1	-0.083	0.2495	1	-0.087	0.2519
5	-0.214	0.0754	5	-0.125	0.0219
10	-0.342	0.0590	10	-0.421	0.1363
15	-0.467	0.0576	15	-0.522	0.0465
20	-0.701	0.1078	20	NC	NC
30	-0.827	0.0290	Average Rate constant		0.1142
Average Rate constant		0.0964	t1/2 (days)		6.07
t1/2 (days)		7.19	CC		-0.9765
CC		-0.9845			

Table 4 Kinetic Analysis of Temephos in the pH 9 Buffer Solution

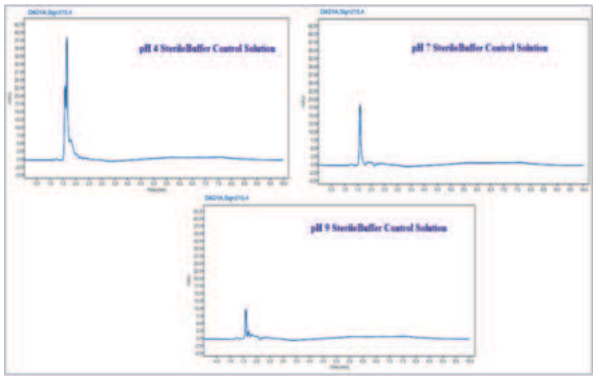

Figure 2. Representative Chromatograms of Buffer Controls in 0th Day Occasion

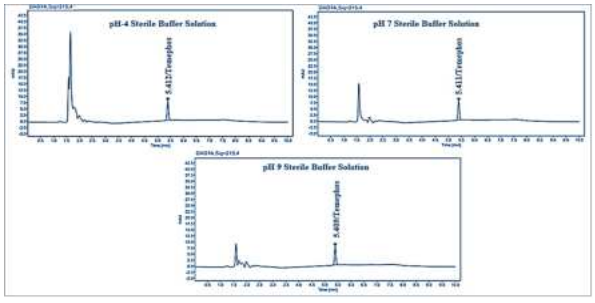

Figure 3. Representative Chromatograms of Temephos in different Buffer Solutions in 0th Day Occasion

CONCLUSIONS

On the basis of these results, aqueous abiotic hydrolysis would be expected to contribute significantly to the degradation of Temephos Technical at pH 4, pH 7, and pH 9. This indicates that the chemical breakdown of Temephos Technical through hydrolysis occurs across a range of acidic to basic aqueous environments, which could have implications for its environmental persistence and efficacy as an insecticide.

REFERENCES

1. Juan Pablo Martínez-Mercado, Adolfo Sierra-Santoyo, Francisco Alberto Verdín-Betancourt, Aurora Elizabeth Rojas-García & Betzabet Quintanilla-Vega (2022) Temephos, an organophosphate larvicide for residential use: a review of its toxicity, Critical Reviews in Toxicology, 52:2, 113-124, DOI: 10.1080/10408444.2022.2065967
2. Dina Satriawan , Wibi Sindjaja , Timmy Richardo, Toxicity of the Organophosphorus Pesticide Temephos, Indonesian Journal of Life Sciences Vol. 01 | Number 02 | September (2019) http://journal.i3l.ac.id/ojs/index.php/IJLS.
3. Vinaya Shetty, Deepak Sanil, and N. J. Shetty, Inheritance Pattern of Temephos Resistance, an Organophosphate Insecticide, in Aedes aegypti (L.), Genetics Research International Volume 2015, Article ID 181872, 6 pages, http://dx.doi.org/10. 1155/2015/181872.
4. Palomino, M., Pinto, J., Yañez, P. et al. First national-scale evaluation of temephos resistance in Aedes aegypti in Peru. Parasites Vectors 15, 254 (2022). https://doi.org/10.1186/s13071-022-05310-x.
5. Mateus Chediak, Fabiano G Pimenta Jr, Giovanini E Coelho, Spatial and temporal country-wide survey of temephos resistance in Brazilian populations of Aedes aegypti, Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. 111(5): 311-321, May 2016.
6. Braga IA, Lima JBP, Soares SS, Valle D. Aedes aegypti resistance to temephos during 2001 in several municipalities in the states of Rio de Janeiro, Sergipe, and Alagoas, Brazil. Mem Inst Oswaldo Cruz. 2004; 99(2): 199-203
7. Yuanjun Guo, Yihui Wang, Shijun Fan, Kaiqi Wang, and Wenhua Han, A Systematic Review of Photolysis and Hydrolysis Degradation Modes, Degradation Mechanisms, and Identification Methods of Pesticides, Journal of Chemistry, Volume 2022, Article ID 9552466, 13 pages https://doi.org/10.1155/2022/9552466.
8. Katagi T. Abiotic hydrolysis of pesticides in the aquatic environment. Rev Environ Contam Toxicol. 2002;175:79-261. PMID: 12206055.
9. Samuel D. Faust & Hassan M. Gomaa (1972) Chemical Hydrolysis of Some Organic Phosphorus and Carbamate Pesticides in Aquatic Environments, Environmental Letters, 3:3, 171-201, DOI: 10.1080/00139307209435465.
10. Muszyński, P., Brodowska, M.S. & Paszko, T. Occurrence and transformation of phenoxy acids in aquatic environment and photochemical methods of their removal: a review. Environ Sci Pollut Res 27, 1276–1293 (2020). https://doi.org/10.1007/s11356-019-06510-2.
11. Simo O. Pehkonen & Qi Zhang (2002) The Degradation of Organophosphorus Pesticides in Natural Waters: A Critical Review, Critical Reviews in Environmental Science and Technology, 32:1, 17-72, DOI: 10.1080/10643380290813444.
12. Liu B, McConnell LL, Torrents A. Hydrolysis of chlorpyrifos in natural waters of the Chesapeake Bay. Chemosphere. 2001 Sep;44(6):1315-1323. DOI: 10.1016/s0045-6535(00)00506-3. PMID: 11513107.
13. K. VALA RAGNARSDOTTIR; Environmental fate and toxicology of organophosphate pesticides. *Journal of the Geological Society* 2000;; 157 (4): 859–876. doi: https://doi.org/10.1144/jgs.157.4.859
14. Cui, Y., Wang, S., Mao, X. *et al.* Hydrolytic Behavior of Novel Pesticide Broflanilide and Its Dissipative Properties in Different Types of Soils. *Bull Environ Contam Toxicol* 111, 8 (2023). https://doi.org/10.1007/s00128-023-03759-9.