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Determination of Insecticide Residues in the Air at the Breathing Zone While Exposure the Long Lasting Insecticidal Net Using Gas Chromatography with Mass Spectrometry - (GC-MS/MS)

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Abstract Long-lasting insecticidal nets (LLIN) are highly effective tools for preventing malaria infection and reducing associated morbidity and mortality [1]. Vector management chiefly through the usage of insecticide inside recurring spraying (IRS) as well as durable insecticidal nets (LLINs). The concept of utilizing insecticide-treated bed nets came during World War II, when Germans, Russian, and US armies began dealing with their combat uniforms of theirs as well as bed nets to safeguard them against vector-borne problems, primarily malaria as well as leishmaniasis [2]. There are many kinds of nets accessible. They differ by size, material, or therapy. The majority of nets are produced from polyester but nets can also be obtainable in cotton, polyethylene, or maybe polypropylene [3,4]. These insecticides are proven to pose really low health consequences to other mammals and humans, but are extremely poisonous to insects and knock them down, while at really low doses [5]. Mosquitoes drawn towards the LLIN due to the attraction of host (Personnel sleeping down the net), on landing the Insecticide present on the nets knockdown and kill the mosquitoes [6]. The health consequences from the LLINs themselves haven't been properly investigated as well as reported in the peer-reviewed scientific literature. A scientifically rigorous, objective, and also a transparent examination of the risks related to LLINs is necessary to give risk-benefit considerations from this promising technological innovation. Pyrethroids present in the air even they are semi-volatile organic compounds. The pesticide inhalation exposures from the bed net were estimated based on indoor Bomann study [7] The level of breathing zone pesticide was estimated this experiment and determined the concentration of active ingredient present in the breathing zone while using the Deltamethrin 1.52 g/kg coated onto filaments Polyester Long Lasting insecticidal Net. The possible daily systemic dose likely to prevail at the breathing zone due to the inhalation of insecticide while sleeping under the treated net is determined. The experiment was conducted in a chamber of size 10 ft x 10 ft x 10 ft (L x B x H). The net sample was fixed in the room following the routine usage pattern. The indoor air was sampled at an interval of 0 hr (5 minutes after experiment initiation), 2 hr, 4 hr, 9 hr, 10 hr, 11 hr, 12 hr and 14 hr. The air sampling was done at a constant flow rate of 5 L/min for 5 minutes (25 L of air). At each sampling occasion the air was suctioned through silica cartridges from four pre-determined locations of the room using suction pump assembly having air rotameters. The experiment was repeated for three days. The residue of Deltamethrin in air was determined for a period of 14 hours in static conditions (no air exchange in the experimental chamber) and dynamic condition at an air exchange rate 0.5 AER/h.

Keywords: *breathing zone, air concentration, LLIN, pesticide, residue, household, malaria, MRM, risk of LLIN, GC-MS, Deltamethrin*

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1. Introduction

The aim of the experiment was to estimate the levels of insecticide residues in the air at the breathing zone while using the long lasting insecticidal treated nets (LLIN). The distribution of LLINs must be systematically accompanied by the provision of info regarding how to hang, utilize and keep them correctly. It's thought that neither LLINs nor by themselves will be completely successful to attain and keep interruption of transmission in hyper-endemic or holo-endemic places. Bed nets have been largely produced for the goal of safeguarding against flies, and mosquitoes along with other biting insects and to safeguard against transmission of diseases including malaria, yellow fever, and dengue fever. Pyrethroids don't quickly fail unless washed or subjected to direct sunlight [8]. The fantastic significance of pyrethroid deposit on Nets arises since the body odour of sleepers attracts human looking for (anthropoliphic) mosquitoes making contact with the net therefore the majority of mosquitoes are killed. Consequently, with prevalent use of medicated nets, the mortality of theirs will be extremely large that a person would anticipate a decrease in mean mosquito grow old and therefore a significant decrease in the population of mosquitoes [8]. The prospective routes of exposure which have to be taken into consideration for danger assessments on all those sleeping under medicated nets are inhalation; dislodge able residues from long lasting insecticidal nets. Among so many trials out there which have calculated the airborne levels of insecticides below medicated nets. [9,10]. The experiment was conducted to determine the concentration of active ingredient present in the breathing zone while using the Deltamethrin 1.52 g/kg coated onto filaments Polyester Long Lasting insecticidal Net. The possible daily systemic dose likely to prevail at the breathing zone due to the inhalation of insecticide while sleeping under the treated net is determined. The net sample was fixed in the room following the routine usage pattern. The indoor air was sampled at an interval of 0 hr (5 minutes after experiment initiation), 2 hr, 4 hr, 9 hr, 10 hr, 11 hr, 12 hr and 14 hr. The experiment was repeated for three days.

2. Materials and Methods

Acetone was supplied by Merck Life sciences, Mumbai. India, Silica Traps were supplied by Agilent Technologies, Inc. Limited, U.S.A. Gas chromatograph GC-2010 plus series fitted with on-column injector and an AOC 20s auto sampler, TQ8040 Mass selective Detector and GCMS solution software supplied by Shimadzu Japan. Column DB-5 MS, (30m length x 0.25mm I.D. x 0.1 µm film thickness supplied by Agilent technologies Inc., Mettler Toledo Analytical Balance Model AG-245 capable of weighing 0.01 mg supplied by Mettler AG, Switzerland. Sartorius analytical balance - Model GP 3202 supplied by Sartorius Mechatronics India Private Limited, Bangalore, India. Rotameters (Flow meter) supplied by Placka instruments, Chennai, Vacuum pumps supplied by M/s. Buchi Rotovapour, Switzerland, Ultra Sonicator supplied by Ultra Instruments, Chennai, India and Anemometer – AM-Y21 supplied by Lutron, Taiwan

2.1. Experiment Setup

The experiment was conducted in a chamber of size 10 ft x 10 ft x 10 ft [Length (L) x Breadth (B) x Height (H)]. The Schematic diagram of standard room (10x10x10 -LBH) for breathing zone concentration was presented in Figure 1 and Figure 2. The net sample was fixed in the room following the routine usage pattern. The indoor air was sampled at an interval of 0 hr (5 minutes after study initiation), 2 hr, 4 hr, 9 hr, 10 hr, 11 hr, 12 hr and 14 hr. The air sampling was done at a constant flow rate of 5 L/min for 5 minutes (25 L of air). At each sampling occasion the air was suctioned through silica cartridges from four pre- determined locations of the room using suction pump assembly having air rota meters. All the sample collection traps are placed at 0.6m height from the floor, the collection points are:

A = Trap-1 located adjacent to the net [10 cm away from the net]

B = Trap-2 located at the center of the net in the room [inside the net]

C = Trap-3 located at corner of the net in the room [outside the net in the room]

D = Trap-4 located at possible breathing zone [inside the net]

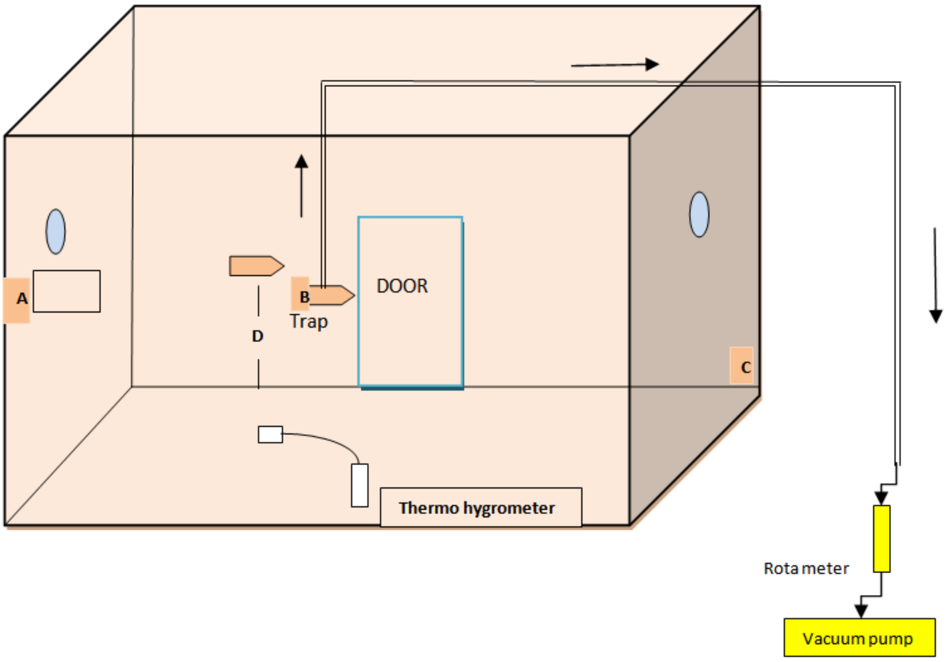


Figure 1. The Schematic diagram of standard room (10x10x10 -LBH) for breathing zone concentration

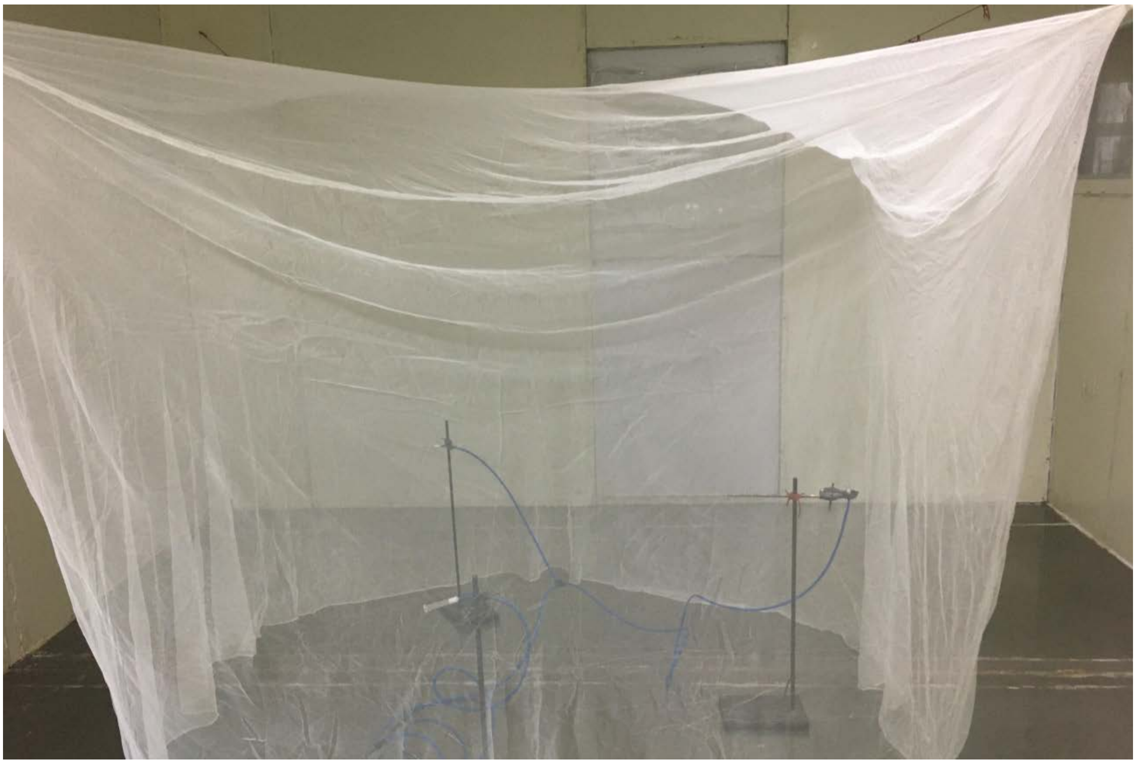


Figure 2. Exposure of the Net in a chamber

Two types of experiments were conducted one under static condition without any air circulation in the room and the other under dynamic condition with air circulation in the room using wall mounted exhaust fan with an air exchange rate of 0.5 AER/h. Each day the net was fixed in the chamber following the routine usage pattern and continued the experiment for 14 hours. End of each day of experiment the net was removed in the chamber and air exchange was provided. The air in the room was sampled in triplicates (control sample) at each point before initiating the experiment. The chamber door was kept closed during the experiment and opened only at the time of trap collection. The study was carried out at ambient room temperature (24.5°C – 26.7°C) and relative humidity (38% - 42%).

2.2. Instrument Conditions

A GC-2010 plus gas chromatograph was connected to a MS TQ 8040 Mass spectrometry system equipped with an auto sampler AOC 20i (Shimadzu, Japan). The column used was a capillary column Agilent technologies Inc., DB-5 MS, (30m length x 0.25mm I.D. x 0.1 µm film thickness). The GC-MS operating condition comprised column temperature (which increased from 40°C held for 1 min at 30°C min-1 to 130°C at 5°C min-1, 250°C at 10°C min-1 and was further increased to 300°C, which was held for 5 min); carrier gas (helium and purity 99.999%, flow rate 1.2 ml min-1); injection port temperature (280 °C); injection volume (1.0 µl); injection mode (split less, purge after 1.5 min ionization voltage, and 70 eV); ion source temperature (230°C); GC-MS interface temperature (280°C). The sample were analysed in Multi reaction monitoring (MRM) mode and the transition are selected in the most abounded daughter ions (for each compound, one quantifying ion and one qualifying ions were selected. When detection is performed by tandem mass spectrometry methods, confirmation of the presence of the analyte requires the observation of a precursor ion plus two structurally significant product ion observed at the same retention time considering the quantifier and confirmatory ion. Two of these product ions were selected: one product ion (MRM-transition) serving for quantitation ion m/z 181.00 →152.00 with 15v of collision energy and the second for confirmation ion m/z 77.00 → 51.00 with 26v of collision energy in a MRM mode of analysis.

2.3. Calibration Standards

Deltamethrin reference standard 10.14 mg was weighed into a 10 mL volumetric flask, added approximately 5 ml of acetone was and kept in ultra-sonicated for homogenization up to content was dissolved and the volume of the flask was brought up to the mark with acetone. The solution was shaken thoroughly and coded as “Deltamethrin standard stock solution”. The concentration of the solution after purity correction was 1012.98 µg/mL. Transferred exactly 9.87 µL of “Deltamethrin standard stock solution” into a 10.0 mL volumetric flask and the content was dissolved and the volume was brought up to 10.0 mL with acetone. The solution was shaken thoroughly and coded as Deltamethrin 1000.0 ng/mL or ppb solution. Calibration solutions were prepared by accurately transferring various amounts of aliquots of stock solution into different 10 mL flasks. The flasks were filled up to their mark with acetone, mixed well and coded as CS1, CS2, CS3, CS4, CS5, CS6, and CS7 and the concentration of the calibrations were 0.1, 0.5, 1.0, 2.0, 5.0, 10 and 20 ng/mL respectively. A small quantity of all calibration solutions were injected to the GC-MS/MS chromatographic system. The linearity of the method was determined by injecting seven linear concentrations of deltamethrin reference standards. A linear curve was plotted for the concentration of standards versus observed peak area, and the correlation coefficient was determined.

2.4. Extraction Procedure

The trapping cartridges collected at different sampling intervals were eluted with 10.0 mL of acetone twice and the solvent was pooled in to a 100.0 mL round bottomed flasks and the sample volume was reduced near to dryness using buchi rota evaparater. Finally each sample was reconstituted with 1.0 mL of acetone. The solution was transferred to vials then injected and analysed using a validated instrumentation method.

2.5. System Precision and Trapping Efficiency

The system precision was determined initially by injecting six times a typical standard solution prepared at 1.0 ng/mL concentration. The solution coded as P1R1 to P1R6. The precision of the method was determined by analyzing the relative standard deviation in the observed area. The recovery from the trapping material was determined by spiking with Deltamethrin standard solution at two fortification levels (LOQ level: 1.0 mL of Deltamethrin 10.0 ng/mL standard solution, 10 x LOQ level: 1.0 mL of Deltamethrin 100.0 ng/mL standard solution). Five replicate determinations were made at each fortification level along with two controls. After fortification, clean air was passed through the trap at the experimental rate volume (5 L/min for 5 minutes), the residues retained in the trap were eluted with 20 mL of acetone and evaporated to near dryness using buchi rota evaporator. Reconstituted the residues in the flask with 1.0 mL of acetone and the sample solutions were filtered through the PTFE syringe filters and analyzed by GC-MS/MS.

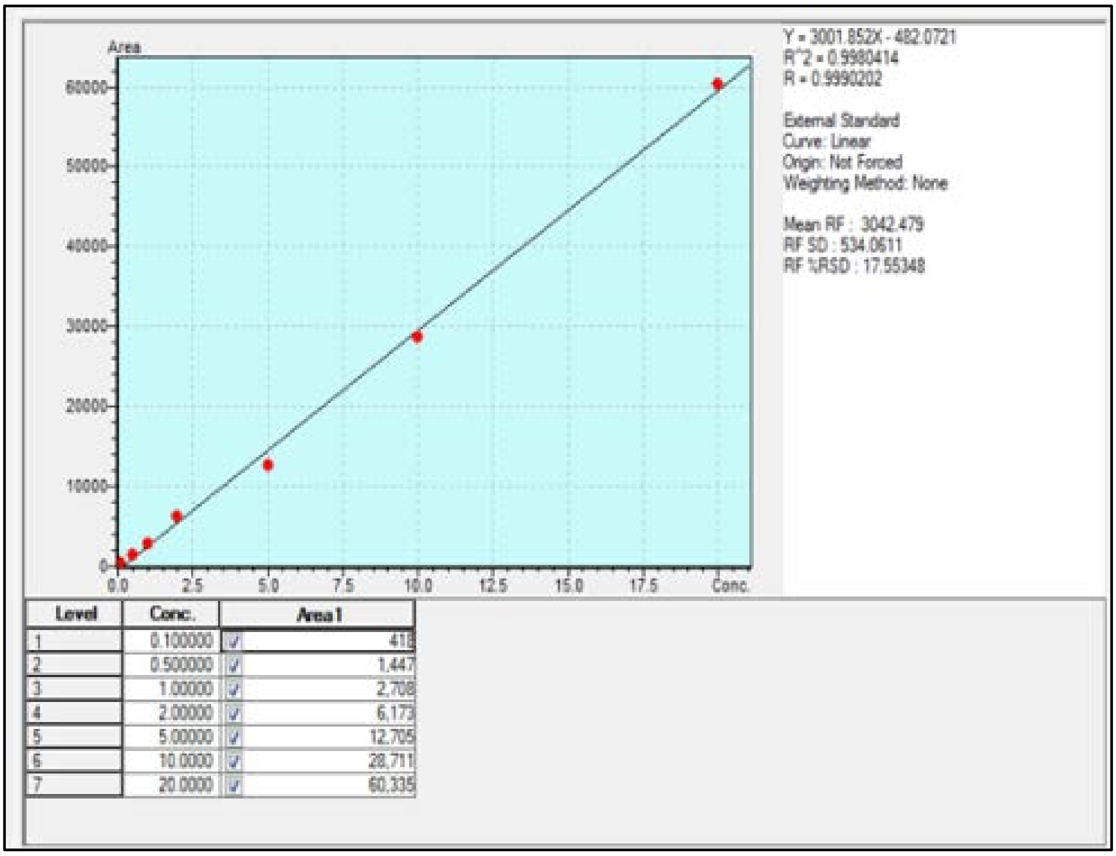
3. Results and Discussion

3.1. Calibration Curve

A plot of the peak area and the concentration of Deltamethrin showed acceptable linearity, as indicated by the correlation coefficient of 0.9980. The results representative calibration curve is presented in Figure 3 and overlaid chromatograms are presented in Figure 4. The limit of detection and limit of quantification for Deltamethrin were established at 0.1 ng/mL and 1.0 ng/mL based on the minimum signal to noise ratio (S/N) 3:1 and 10: 1.

3.2. System Precision and Trapping Efficiency

The percentage relative standard deviation among six replicate injections of 1.0 ng/mL concentration solution was 1.08 ng/mL for Deltamethrin. This indicates an acceptable system precision. The results are presented in Table 1. The overall assay trapping efficiency of standard at LOQ level and at higher level was 91.44 and 95.86% respectively. The standard deviation at LOQ level and higher level were 1.88 and 1.00 an acceptable method of analysis. The data presented in the Table 2.



Y = 3001.852X - 482.0721
R^2 = 0.9980414
R = 0.9990202

External Standard
Curve: Linear
Origin: Not Forced
Weighting Method: None

Mean RF : 3042.479
RF SD : 534.0611
RF %RSD : 17.55348

Level	Conc.		Area1
1	0.100000	✓	418
2	0.500000	✓	1,447
3	1.00000	✓	2,708
4	2.00000	✓	6,173
5	5.00000	✓	12,705
6	10.0000	✓	28,711
7	20.0000	✓	60,335

Figure 3. Calibration curve

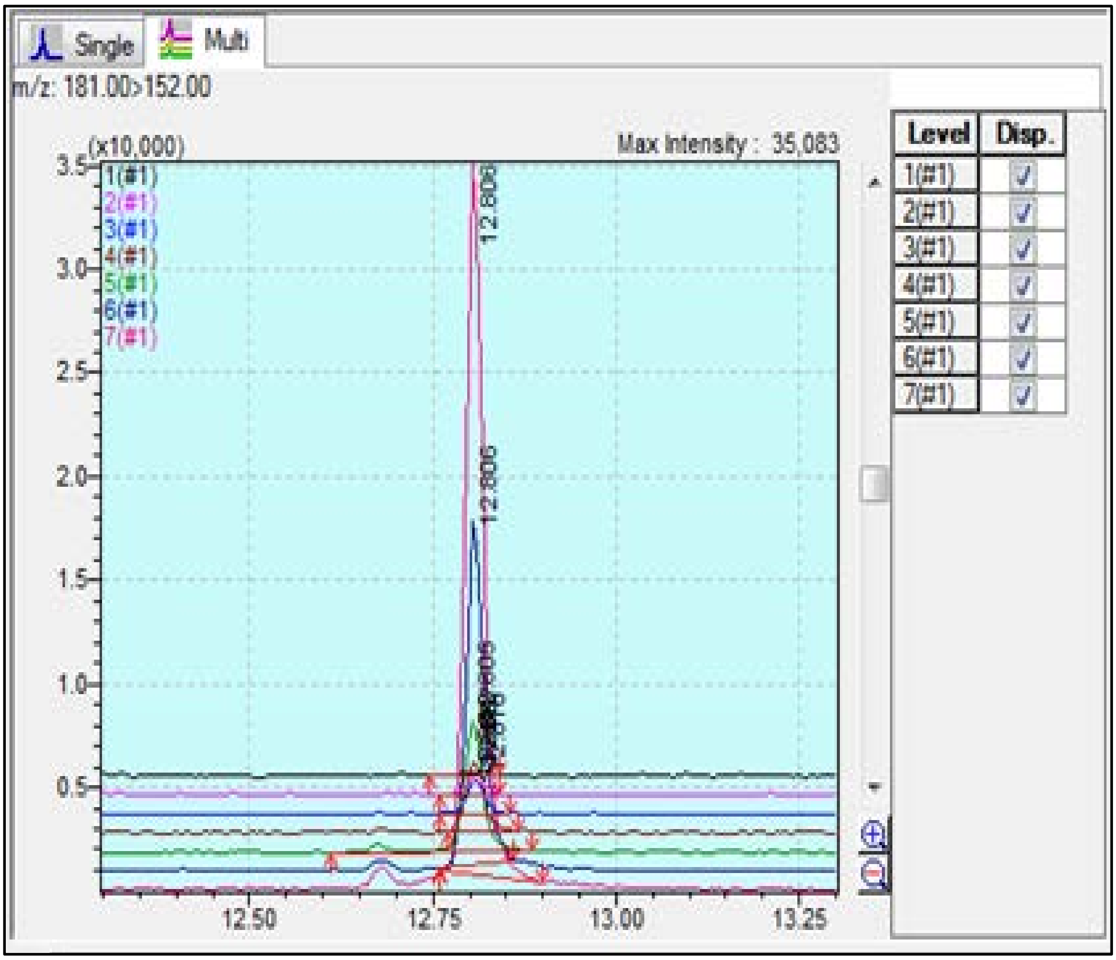


Figure 4. Overlaid chromatograms

Table 1. System Precision - Deltamethrin

Code	Peak Area (Counts*Sec)	Slope	Intercept	Concentration of injected sample (ng/mL)	Average (ng/mL)	SD	RSD (%)
P1R1	3564	2672.83	657.40	1.09	1.08	0.02	1.66
P1R2	3514	2672.83	657.40	1.07			
P1R3	3604	2672.83	657.40	1.10			
P1R4	3578	2672.83	657.40	1.09			
P1R5	3496	2672.83	657.40	1.06			
P1R6	3488	2672.83	657.40	1.06			

Table 2. Trapping efficiency - Deltamethrin

Sample Code	Peak Area (Counts*Sec)	Slope	Intercept	Initial Volume	Final Volume	Recovered concentration (ng/mL)	Recovery (%)	Average	Standard Deviation
CS4	3852	2672.83	657.40	-	-	-	-	-	-
S0R1	-	2672.83	657.40	-	-	-	-	-	-
S0R2	-	2672.83	657.40	-	-	-	-	-	-
S1R1	781	2672.83	657.40	20.00	1.00	0.92	92.49	91.44	1.88
S1R2	782	2672.83	657.40	20.00	1.00	0.93	93.23		
S1R3	778	2672.83	657.40	20.00	1.00	0.90	90.24		
S1R4	776	2672.83	657.40	20.00	1.00	0.89	88.74		
S1R5	781	2672.83	657.40	20.00	1.00	0.92	92.49		
S2R1	1955	2672.83	657.40	20.00	1.00	9.71	97.10	95.86	1.00
S2R2	1941	2672.83	657.40	20.00	1.00	9.60	96.05		
S2R3	1923	2672.83	657.40	20.00	1.00	9.47	94.70		
S2R4	1954	2672.83	657.40	20.00	1.00	9.70	97.02		
S2R5	1936	2672.83	657.40	20.00	1.00	9.57	95.67		

3.3. Deltamethrin Residues in the Air at the Breathing Zone

The residues of Deltamethrin in air were determined for a period of 14 hours in static (no air exchange in the experimental chamber) and Dynamic Conditions. The experiment was repeated for three days and on each day and on each time point collected the air samples. The Average contentment of residue for three days was presented in the Table 3 and the representative residual graph was presented in Figure 5.

Table 3. Deltamethrin content in air

Deltamethrin content in air (µg/m3) (Average content of Day 1, Day 2 and Day 3)		
Time (Hour)	**Deltamethrin (Static)**	**Deltamethrin (Dynamic)**
0	0.044	<LOQ
2	0.058	<LOQ
4	0.073	0.038
9	0.089	0.045
10	0.102	0.051
11	0.160	0.055
12	0.207	0.059
14	0.258	0.063

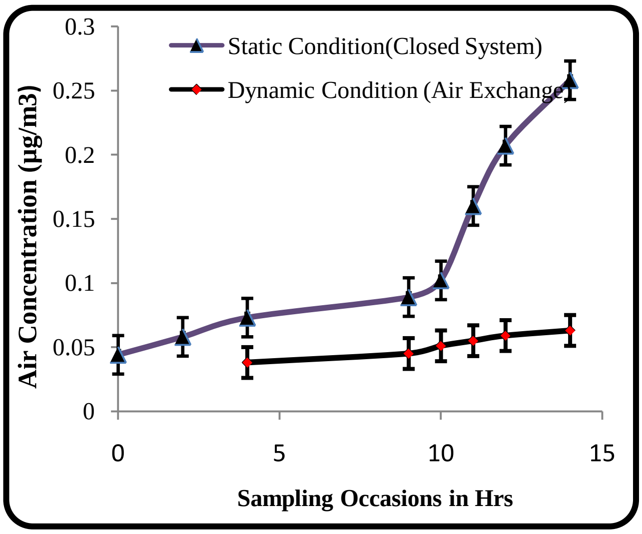


Figure 5. Air Residual trends for 14 Hours

4. Conclusion

The possible daily systemic dose prevail at the breathing zone due to the inhalation of insecticide while sleeping under the treated net was determined using the deltamethrin coated Long-Lasting Insecticidal Net (LLIN). The experiment was conducted under static and Dynamic conditions. The three days average residue concentration of static condition at 0.0 hr: 0.041 $\mu g/m^3$ at Trap A, 0.041 $\mu g/m^3$ at Trap B, 0.045 $\mu g/m^3$ at Trap C, 0.047 $\mu g/m^3$ at Trap D and at 14 hr: 0.256 $\mu g/m^3$, 0.260 $\mu g/m^3$, 0.255 $\mu g/m^3$, 0.261 $\mu g/m^3$ at Trap A, B, C and D, respectively. The three days average residue concentration of Dynamic condition at Deltamethrin at 0.08 hr: < LOQ at Trap A, B, C, D, respectively and at 14 hr: 0.063 µg/m3, 0.064 µg/m3, 0.063 µg/m3, 0.063 µg/m3 at Trap A, B, C and D, respectively. The residues level of the breathing zone for the dynamic conditions are very low when compared with static condition.

Acknowledgements

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