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Estimation of the Levels of Meperfluthrin Pesticides in the Body Organelles of Albino Rats through Inhaling Smoke Produced by Mosquito Coils

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ABSTRACT

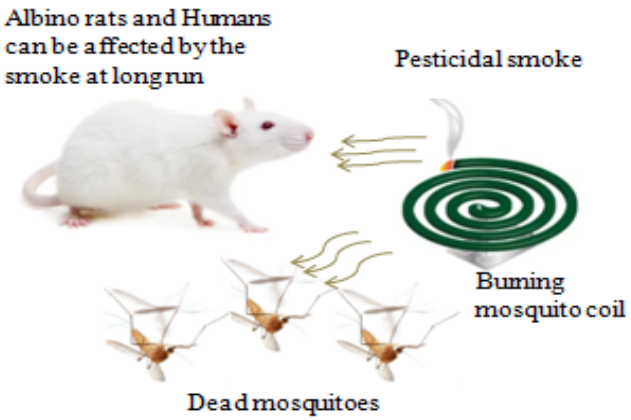
The domestic use of mosquito coils as pesticides has been increasing over the years to repel and kill mosquitoes as well as other household insects especially in the rural areas. Unfortunately, this could lead to excessive accumulation of pesticides in the body organs due to continuous inhaling of the chemical compounds over a long period of time. And this could result in health issues that include various kinds of organelle cancers, congenital disabilities etc. For this reason, there is a need to determine the levels of Meperfluthrin pesticides from mosquito coil smoke. Stock and standard solutions of the analyte were prepared and used for the calibration of the instrument. Later on, the technique of quick, easy, cheap, effective, rugged and safe (QuEChERS) coupled with dispersive solid phase extraction (dSPE) was used for sample preparation. Then, the calibrated UV-visible spectrophotometry instrument was used for the determination of the pesticide residues in the organelle samples of albino rats; blood (SRBL), lungs (SRLU), kidney (SRKI) and liver (SRLI) after a periodical inhalation of smoke produced by mosquito coils. Similarly analysis was carried out on the organelle samples of the unexposed (blank) albino rats. Eventually, the results (Average ± Standard deviation) obtained (per 28 days) showed that the analyzed samples were accumulated with the targeted analyte; SRBL (199 ± 0.03 mg/kg), SRLU (321 ± 0.01 mg/kg), SRKI (129 ± 0.05 mg/kg) and SRLI (564 ± 0.07 mg/kg) after deducting the concentration from the blank animal organs, respectively. Therefore, these justify the continuous accumulation of the targeted pesticides over a period of time, which can result in health issues since the average determined concentration of pesticides residue (11 mg/kg per day) was above the maximum daily residue limits (MRLs) of 5 mg/kg documented by Food and Agriculture Organization of the United Nations, and World Health Organization.

Keywords: Albino rats, Meperfluthrin, Mosquito, pesticides, QuEChERS-dSPE

INTRODUCTION

Environmental mismanagements have contributed to the major issues affecting population increase in the rural areas especially in the regions of African communities such as Nigeria (Uthman *et al.*, 2016). However, some of these issues include over population of insects (vectors) such as mosquitoes, which have negative effects on human health, treacherously causing malaria fever that leads to the death of million people over the years, especially in young children (Nmadu *et al.*, 2015). Based on this reason, various ways have been used to prevent the bite of anopheles mosquitoes such as the use of mosquito repellants, nets and pesticides. The pesticides are usually applied in the form of liquids, aerosol sprays and smoke produced by mosquito coils (Hogarh *et al.*, 2016). The mosquito coils mainly consist of insecticides that are capable of smoldering and burning without flame. And the mosquito coils usually composed of chemical materials such as synthetic pyrethroid (pesticides), binders, organic fillers, dyes and other additives (Hassan *et al.*, 2019). It is the most widely known effective mosquito repellent used in Asia and Africa by the low-income communities because of its low price, availability and very easy to use as insecticide (Debboun & Strickman, 2013). Unfortunately, the high rate at which mosquito coils is being used by the populous could leads to excessive accumulation of the vital organs of the body that include lung, blood, liver and kidney with the smokes of mosquito coils through inhalation. Meanwhile, the harmful and health threatening chemical compounds in the smoke of mosquito coils include carbon-black, polycyclic aromatic hydrocarbons (PAHs), heavy metals and pesticides residue (Abdulra'uf & Lawal, 2020; Doro *et al.*, 2021; Elehinafe *et al.*, 2022; Koki *et al.*, 2018; Lawal *et al.*, 2016; Lawal & Wong, 2021; Nassar & Ismail, 2021). Consequently, these could result in many health issues at a long run that include cancer risks in both adults and children such as Adenocarcinoma, Leukemia, *Hepatocellular carcinoma, Renal cell carcinoma as well as* congenital disabilities (Madhubabu & Yenugu, 2017). Therefore, organelle parts of albino

rats can be used to estimate the concentration of the inhaled pesticides residue from mosquito coils after exposure for a certain period of days as illustrated in Scheme 1.



Scheme 1: Dead mosquitoes killed by smoke of Mosquito coil and inhaled by an Albino rat

Objectively, the concentration of the inhaled residue of pesticides can be determined in the vital organelle parts of the studied animals occasionally in order to address the health issues. However, the present study focuses on the analysis of Meperfluthrin pesticides **(**$C_{17}H_{16}C_{l2}F_4O_3$**)** residue as the most active pesticides ingredient in mosquito coils. Meperfluthrin (analyte) has an IUPAC name of [2,3,5,6-tetrafluoro-4-(methoxymethyl)phenyl]methyl(1*R*,3*S*)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate as structurally illustrated (Fig. 1). Thus, the analyte residues will be determined in the blood (SRBL), lungs (SRLU), kidney (SRKI) and liver (SRLI) samples of albino rats after periodic inhalation of smoke produced by mosquito coils.

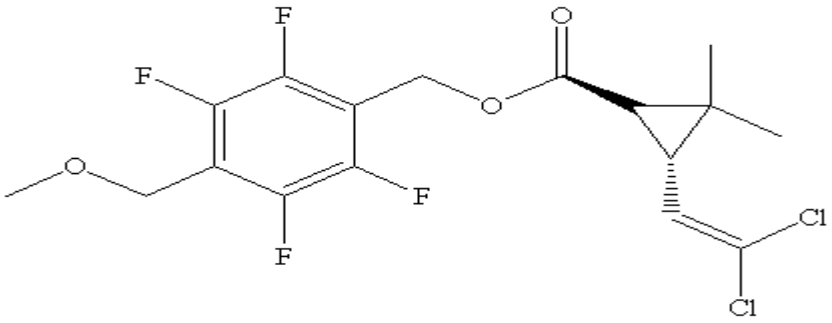


Fig. 1: The structure of Meperfluthrin pesticides

Moreover, the modified quick, easy, cheap, effective, rugged and safe (QuEChERS) coupled with coupled with dispersive solid phase extraction (dSPE) techniques was used for the sample preparation because of its effectiveness over the other conventional methods such as liquid-liquid extraction (LLE), which was found to be less effective (Aliu *et al.*, 2023; Lawal, 2018; Lawal & Abdulra'uf, 2020; Lawal & Haliru, 2021; Lawal & Kah, 2021; Lawal & Koki, 2019; Lawal *et al.*, 2019; Lawal *et al.*, 2018a, 2018b). Later on, quantitative analysis of the extracted analytes was conducted using UV-visible spectrophotometry because of its easy availability and operational technical advantage over the other quantitative instruments. Therefore, it is hoped that this study will create awareness and guide against excessive usage of such products by the entire populous community and also serve as a reference guide for future studies.

MATERIALS AND METHODS

Sampling and treatments

A pack of mosquito coils containing Meperfluthrin pesticides was purchased from the Katsina central market, Katsina State, Nigeria. Subsequently, the technique used by Vichare *et al.* (2010) was adopted for preparation of the 100 mg/kg stock solution; an estimated 0.025 g of the ground mosquito coils was transferred into a 500 mL conical flask before dissolving with a 250 mL of methanol, which served as the stock solution. The stock solution was filtered and diluted into 20, 40, 60, 80 and 100 mg/kg standard solutions respectively with estimated volumes of methanol (Lawal & Koki, 2019). The standard solutions were preserved in sample bottles and placed in a refrigerator before using them to calibrate the UV-visible spectrophotometry instrument (Lawal *et al.*, 2020; Lawal *et al.*, 2021; Yarima *et al.*, 2021).

Instrumentation

The UV-visible spectrophotometer single beam with matched quartz cell 1.0 cm and T60 model was used to analyze the blank and the prepared samples after the instrument was calibrated using the prepared stock/standard solutions of Meperfluthrin pesticides. All analyses were carried out in triplicates to determine the mean (average) and standard deviations.

Samples collection after inhaling smoke of mosquito coils by the experimental animals

A total of ten (10) adult albino rats with an average weight of 425 g were purchased from the Animal-House of Umaru Musa Yar'adua University Katsina, Nigeria. The rats were divided into 2 groups. However, each of the five (5) rats were kept in cages and named Group A and B, respectively. Each of the groups was placed in two (2) separate small rooms in an Animal-House with similar ventilation system. Later on, Group A rats were exposed with the smoke released by two individually burned mosquito coils (one after the other) for nine (9) hours, daily over a period of twenty-eight (28) days. While, Group B (blank) were kept in a separate room and were not exposed to the smoke released by the burned mosquito coils for the same period of time. Subsequently, each of the animals from each group was sacrificed and its organs (lungs, kidney and liver) were harvested using dissection equipment and preserved in separate labeled sample bottles. Also, the blood samples were individually collected using a syringe and preserved in the labeled sample bottles. Then, all the 40 analytical samples (4 samples of blood, lungs, kidney and liver for the 5 exposed and 5 unexposed animals) in the labeled sample bottles were preserved in a refrigerator before extraction of the pesticides residue using the QuEChERS-dSPE techniques.

Samples preparation

The validated method of QuEChERS-dSPE reported by Lawal *et al.* (2018a) as well as Lawal and Koki (2019) was adopted for the sample extraction of the 40 analytical samples. The method started by transferring 5 mL blood sample for one of the blank group of animals into a 15 mL centrifuge tube and 4.5 mL of acetonitrile was added. Then, 1.8 g of anhydrous $MgSO_4$ and 0.45 g of NaOAc salts were added. The mixture was vortexed for l min and centrifuged at 4000 rpm for 2 min. Afterward, the d-SPE extraction cleanup was carried out by transferring 1.5 mL supernatant from the QuEChERS extraction into 2 mL centrifuge tube containing 0.2 g $MgSO_4$. The tube was vortexed and centrifuged (4000 rpm) for 5 min. The extract was analyzed with UV-visible spectrophotometry instrument at the wavelength of 280 nm (Alonso *et al.*, 2014) and the absorbance readings were recorded. Note that, the method above was similarly used on the remaining analytical samples. Even though, the lungs, livers and kidneys samples were ground and homogenized before carrying out their respective sample preparations to increase their extraction processes. Also, the residual concentrations (mg/kg) of the targeted analyte were estimated using the linear equation (equation 1) of the graph's calibration curve.

$$y = 0.001x + 0.041 \quad (1)$$

Where, y = Absorbance reading of the instrument,
X = Concentration

RESULTS AND DISCUSSIONS

The prepared standard solutions were analyzed and the average absorbance readings of the UV-visible instrument were used for the construction of the calibration curve after deducting out the blank reading as shown in Table 1.

Table 1: The instrumental readings of standard solutions used for calibration curve

Standards	ABS 1	ABS 2	ABS 3	Average ABS	(Average ABS – Blank) ± STDEV
Blank	0.548	0.548	0.548	0.548	X
20 mg/kg	0.627	0.625	0.624	0.625	0.077 ± 0.002
40 mg/kg	0.658	0.657	0.656	0.657	0.109 ± 0.001
60 mg/kg	0.689	0.6869	0.686	0.687	0.139 ± 0.002
80 mg/kg	0.718	0.717	0.717	0.717	0.169 ± 0.001
100 mg/kg	0.778	0.758	0.748	0.761	0.213 ± 0.015

KEY: ABS, UV-visible absorbance reading; STDEV, standard deviation

In the first place, the use of the 280 nm wavelength for the analysis of Meperfluthrin pesticides using UV-visible spectrophotometry instrument is in accordance with report of Alonso *et al.* (2014) and Jain *et al.* (2010) for the analysis of similar pesticide compounds. However, the absorbance readings in Table 1 were satisfactory because it shows that the absorbance results increase as the concentration of standard solutions increases. Even though, the blank sample that

composed of methanol (diluting solvent) alone was analyzed initially and the average absorbance readings was deducted from the average absorbance readings of the standard solutions, respectively to minimized the instrumental error towards the targeted analyte and this agrees with the documentation of Palanikumar *et al.* (2014).

Similarly, Table 2 and 3, respectively shows the average and standard deviations of the instrumental absorbance readings as well as the estimated residual concentrations (mg/kg) of targeted analytes in the organelle samples of blank and exposed animals, respectively for the period of 28 days.

Table 2: The average & standard deviations of absorbance readings of the blank samples and the determined residual concentrations (mg/kg per 28 days)

SAMPLES	ABS Group B-1	ABS Group B-2	ABS Group B-3	ABS Group B-4	ABS Group B-5	Average ABS Group B	Average Conc. Group B (mg/kg) ± STDEV
Blood	0.421	0.364	0.3925	0.3925	0.3925	0.3925	352 ± 0.03
Lungs	0.421	0.401	0.411	0.411	0.411	0.411	370 ± 0.01
Kidney	0.852	0.683	0.7675	0.7675	0.7675	0.7675	727 ± 0.04
Liver	0.66	0.897	0.7785	0.7785	0.7785	0.7785	738 ± 0.05

KEY: ABS, UV-visible instrumental absorbance reading; STDEV, standard deviation; Conc., concentration

Table 3: The average & standard deviations of instrumental readings of the exposed samples and the residual concentrations (mg/kg per 28 days)

SAMPLES	ABS-Group A-1	ABS-Group A-2	ABS-Group A-3	ABS-Group A-4	ABS-Group A-5	Average ABS-Group A-1-5	Average Conc. Group A-1-5 (mg/kg) ± STDEV
Blood	0.62	0.563	0.5915	0.5915	0.5915	0.5915	551 ± 0.02
Lungs	0.742	0.722	0.732	0.732	0.732	0.732	691 ± 0.01
Kidney	0.981	0.812	0.8965	0.8965	0.8965	0.8965	856 ± 0.06
Liver	1.224	1.461	1.3425	1.3425	1.3425	1.3425	1302 ± 0.08

KEY: ABS, UV-visible absorbance reading; STDEV, standard deviation; Conc., concentration

Meanwhile, the average absorbance readings for each sample organelles of the five animals from both blank and exposed group were successfully estimated from the equation of the calibration curve. Eventually, the average residues of Meperfluthrin pesticides were determined in all the analyzed organelle samples and the average actual concentrations were estimated by deducting the average concentration of blank (Group A-1-5) from the average concentration of the exposed (Group A-1-5) group for a period of 24 hours as well as 28 days, respectively as illustrated in Fig. 2 and presented in Table 4.

Table 4: The residual concentration of the targeted analyte in the analyzed samples

SAMPLES	Residual Concentration (mg/kg)/day	Residual Concentration (mg/kg)/28 days ± Standard deviation
Blood	7	199 ± 0.03
Lungs	11	321 ± 0.01
Kidney	5	129 ± 0.05
Liver	20	564 ± 0.07
Average residual intake (mg/kg)/day	11	-

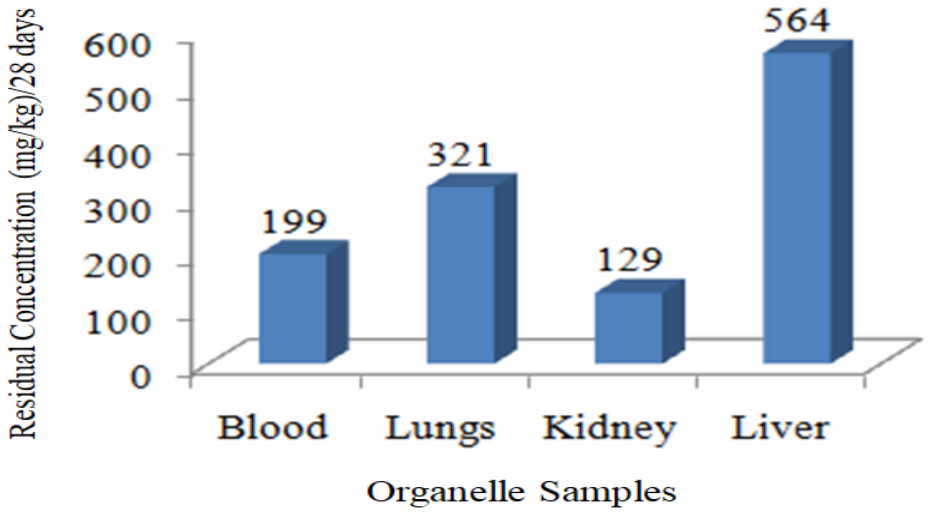


Fig. 2: The residual concentration of the targeted analyte in the analyzed samples

The liver sample accumulated the highest residues (564 ± 0.07 mg/kg) of the targeted analyte for 28 days (20 mg/kg per daily intake). This maybe because it is a store for much kind of materials that include glucose, iron, copper etc. It is also known to detoxify toxic substances in the body. That is the reason most toxic material ingested into the body passes through the liver for detoxification process (Vichare *et al.*, 2010). This agrees with the report of Nassar and Ismail (2021), which shows that high concentration of PAHs were determined in the liver of albino rats after exposure to mosquito coils containing PAHs.

The lungs are the second most accumulated organ with the pesticide residue (321 ± 0.01 mg/kg) for the period of 28 days with an intake of 11 mg/kg per day. This may occur as a result of the initially and continuous deposits of smokes containing the Meperfluthrin pesticides in the lungs tissue by inhalation process before circulating into the blood streams through the alveoli as documented (Song *et al.*, 2019).

The blood is the third most accumulated organ, which contained 199 ± 0.03 mg/kg of pesticides residue for the 28 days of exposure to smoke of mosquito coils. However, this is because the blood constitutes about 8% of the weight of an animal that is meant for transportation of food and waste materials in the body as agreed by El-Nahhal and Radwan (2013).

The least concentration of Meperfluthrin pesticides residue (129 ± 0.05 mg/kg) determined was found in the organelle sample of the kidney. However, since the kidney is known for capability in removing wastes, acids and extra fluid from the body to maintain minerals, salts and water balance. Thus, there is a possibility of removing contaminants such as pesticides residue, which also could reduce the concentration absorbed at long run through urination as accorded by Ndonwi *et al.* (2019).

Therefore, the overall average daily residual intake (11 mg/kg) of Meperfluthrin pesticides determined is above the average daily permissible intake of 5 mg/kg for most of the fresh fruits and vegetables as documented by the International Food Standards with the support of Food and Agriculture Organization of the United Nations, as well as World Health Organization (FAO/WHO, 2023).

CONCLUSION

The estimation of the concentration levels of Meperfluthrin pesticides in the body organelles of albino rats through inhaling smoke produced by mosquito coils was successfully carried out. Therefore, the results obtained justify the continuous accumulation of the targeted pesticides from mosquito coils in the body through inhalation over a long period of time. This can eventually result in health issues since the overall average daily intake residual concentration (11 mg/kg per day) of Meperfluthrin pesticides determined was above the maximum daily residue limits of 5 mg/kg for most of the fresh fruits and vegetables.

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