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COMPARATIVE ANALYSIS OF MORPHOLOGICAL AND HISTOLOGICAL IMPACT ON THE RESPIRATORY TRACT OF ALBINO MICE EXPOSED TO ALLETHRIN-BASED VAPORIZER FUMES VS MOSQUITO COIL SMOKE

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Abstract
Mosquito repellent products release chemical compounds into the atmosphere, leading to concerns regarding their potential impact on individuals who inhale these compounds during usage. In this study, the effects of allethrin exposure, a common ingredient in mosquito repellents such as coils and liquid vaporizers, were examined on the respiratory health of mice. The research assessed alterations in body weight. An animal experiment involved 42 mice in three groups: Control, Liquid Vaporizer, and Mosquito Coil. Their weights were measured at the beginning and end of an 8-week study. Trachea and bronchi, including lungs, were examined grossly. SPSS was used for analysis. The study revealed that exposure to liquid vaporizer and mosquito coil emissions led to changes in mice's body weight, lung color, texture, and the presence of eroded patches. These effects were most pronounced in the Mosquito Coil group, while the Control group remained unaffected. Statistical analysis confirmed the significance of these differences, emphasizing the potential harm of long-term insecticidal exposure. The evaluation of allethrin, a common mosquito repellent component, on respiratory health revealed significant adverse effects.

Introduction
In regions prone to mosquito-borne diseases, the implementation of mosquito repellent measures has become a widespread practice, aimed at safeguarding public health. Mosquitoes, as carriers of various diseases such as malaria, dengue, ZIKA virus, chikungunya, yellow fever, and Congo virus, pose a global health threat (Benelli and Mehlhorn, 2016). However, regions characterized by hot and humid climates, such as the Indo-Pak subcontinent, are particularly susceptible to the relentless spread of these disease vectors (Dhimal et al., 2021). In such areas, the convergence of favorable breeding conditions stemming from optimal temperatures and moisture levels exacerbates the menace of these biting insects (Ghosh and Ghosh, 2020).

The advent of synthetic pyrethroids has revolutionized mosquito repellent technologies, transitioning from traditional coil and powder formats to sophisticated devices such as electrical mats, vaporizers,

aerosol sprays, and lotions (Zhan et al., 2020). Among the prominent synthetic pyrethroid analogs that have gained commercial prominence as active ingredients in diverse indoor insecticides, notable examples include Esbiothrin, Dimefluthrin, Meperfluthrin, Fenvalerate, Metofluthrin, Butylated hydroxytoluene (BHT), and Deltamethrin (Matsuo, 2019).

Among the various methods employed, two commonly used strategies include the use of allethrin-based vaporizers and mosquito coils (Abdulla Al-Mamun et al., 2017). In the Pakistani mosquito fauna, including species such as Culex, Aedes, Anopheles, and Mansoni, susceptibility to d-trans-allethrin is well-documented (Singh et al., 2023). Notably, the highest efficacy of this compound is observed against Aedes mosquitoes, with effectiveness rates ranging from 50% to 80% (Baldacchino et al., 2015). These interventions offer effective means of deterring disease vectors, yet their implications on human health, specifically the respiratory system, have garnered growing attention.

Mosquito repellent products release chemical compounds into the atmosphere, leading to concerns regarding their potential impact on individuals who inhale these compounds during usage (Li et al., 2016). The respiratory system, a complex and vital component of human anatomy, represents a key area of interest when assessing the potential health effects associated with the inhalation of allethrin-based vaporizer fumes and mosquito coil smoke (Alabi et al., 2019).

Vaporizers, integral to many households, consist of three key components: an electric plug-in head, a heating coil, a transmission graphite rod, and a liquid-containing bottle or container. This liquid comprises a blend of three distinct chemicals: a fragrance, a stabilizing agent, such as butylated hydroxytoluene (BHT), and a repellent like d-trans-allethrin, fenvalerate, or esbiothrin (Koluman et al., 2016). BHT serves to prevent the oxidation or hydrolysis of the liquid, while the fragrance functions as an indicator of vapor dispersion. This chemical mixture is often diluted with a medium like kerosene distillate to increase the volume for effective dispersion. Regrettably, despite their widespread use, these synthetic repellents pose health risks to humans, ranging from minor allergies to potentially carcinogenic effects (Khater et al., 2019).

This research aims to provide a comparative analysis of how exposure to allethrin-based vaporizer fumes and mosquito coil smoke affects the respiratory tracts of albino mice. The study also examined both macroscopic (visible) changes and microscopic alterations within the respiratory system. The critical importance of effective mosquito control measures, which extends to the evaluation of the health impact of commonly used mosquito repellent products. In this study, we delved into the specific respiratory health implications of allethrin-based vaporizer fumes and mosquito coil smoke exposure, shedding light on potential risks associated with these prevalent mosquito control methods. Chemically identified as (±)-3-allyl-2-methyl-4-oxocyclopent-2-enyl(±) cis,trans-chrysanthemate, allethrin exists in two isomeric forms, cis and trans, with d-trans-allethrin being its prevalent commercial variant today. Its organic formula is represented as $C_{19}H_{26}O_3$, with a relative molecular weight of 302.41 g/mol. These chemical attributed define allethrin, a compound integral to the composition of mosquito repellent products and central to our investigation of its impact on respiratory health.

Methodology

Study Design:

This study employed an animal experimental design.

Study Settings:

- Animals were housed and maintained in the Experimental Research Laboratory (Animal House) of the Postgraduate Medical Institute, Birdwood Road, Lahore.

- Tissue sectioning and mounting procedures were conducted in the Histology Laboratory of the Anatomy Department at KEMU, Lahore.
- Micrometry and photography were performed in the Histopathology Laboratory, KEMU, Lahore.

Sample Size

The sample size of 42 albino mice (14 in each group) was determined based on the following parameters:
Significance level (α): 5%
Power of the test (1-β): 90%
Expected mean value in the control group (μ0): 5.50 ± 0.44
Expected mean value in treated groups (μa): 4.76 ± 0.21
The formula used for sample size estimation was:
$n = 2\sigma^2 (z1-\alpha + z1-\beta)^2 / (\mu0 - \mu a)^2$
where σ^2 = Variance (0.4225), z1-α = Confidence level (95% = 1.96), z1-β = Power of test (90%), μ0 = Population mean 1 (5.5), μa = Population mean 2 (4.76).

Processing and Methods

A total of 42 active adult Swiss albino mice, of either sex, weighing 25-40 grams and aged 8 to 12 weeks, were acquired. The mice were randomly divided into three groups, each containing 14 animals, using a lottery method based on simple random sampling
The groups were labeled as follows:

1. **Control group (CG)**: Exposed to normal atmospheric air with no inhalational chemical exposure for 8 weeks.
2. **Liquid Vaporizer group (LVG)**: Subjected to inhalational exposure to Allethrin-based Liquid Vaporizer for 8 hours daily, 6 days a week, over an 8-week period.
3. **Mosquito Coil group (MCG)**: Exposed to inhalational exposure to Allethrin-based Mosquito Coil for 8 hours daily, 6 days a week, over 8 weeks.

Each group was placed in separate fiberglass chambers within the Animal House of the Postgraduate Medical Institute, Birdwood Road, Lahore. The chambers' dimensions were 90cm × 60cm × 60cm, with one-half of the roof made of wire mesh for ventilation.

A smaller wire mesh box (15cm × 15cm × 5cm) was positioned under the closed roof area next to the wall in Chambers 2 and 3, designated for LVG and MCG, respectively. This box contained Mortein liquid vaporizer in Chamber 2 and Mortein mosquito coil in Chamber 3, preventing the mice from disturbing it while activated.

The chambers were placed in separate rooms near ventilation points to ensure that fumes from one chamber did not reach the others or affect the rest of the animals in the Animal House.

Preparations

Mortein liquid vaporizer (Peaceful Night), a Reckitt Benckiser product, was purchased from the local market. It contains 3% d-trans-Allethrin by weight in a 60ml bottle of distillate volume. For the experiment, a heating head plug-in and three refills were obtained. One refill was connected to the heating head, with the other kept for future use. Each refill, when used for 8 hours daily with consistent ventilation, was estimated to last around 20-22 days. To facilitate the experiment, an electrical multi-plug extension with a lengthy wire was acquired and placed inside the smaller wire mesh box in Chamber 2. The liquid vaporizer was then connected to this extension's socket for operation. Figure 2

Mortein All-out Mosquito Coils, product of Reckitt Benckesir, was purchased from local market. It contains about 0.3% by mass of coil, d-trans-allethrin Figure 1. chamber 1 was used for coils and each coil took 7-8 hours for complete burning.

Total Body Weights Measurement:

- Initial body weights of experimental animals were recorded on day zero using a Digital weighing Machine at the PGMI animal house.
- Final body weights were measured on the 61st day of the experiment, again using the Digital weighing Machine at the PGMI animal house.
- After the final body weight measurement, euthanasia was administered, starting with morphine (0.3 to 0.5 mg/kg) for analgesia and followed by intraperitoneal injection of sodium pentobarbital (45 mg/kg).
- The animals were immobilized and placed in a supine position on a dissection board.
- A vertical incision was made from the pharynx to the xiphisternum, providing access to the tracheobronchial structures up to the hilum.
- The trachea was excised from the larynx down, and lymph nodes and nearby nervous structures were removed.

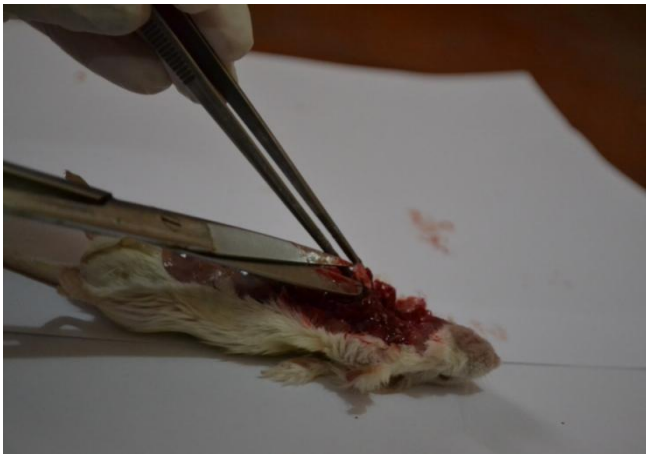


Figure 1 *Mice disection*

- The total weight of the trachea and bronchi, including paired lungs, was measured using a digital weighing machine.
- Thorough cleansing with saline solution was performed.
- The trachea and bronchi up to the hilum were isolated for histological examination.
- Gross inspections assessed color, texture, erosions, sponginess, and congestion, with comprehensive photographic documentation and tabulation.

Figure 2 *Lung appearance*

Data Analysis

SPSS v26.0 analyzed histomorphological data. Quantitative variables were represented via box plots, with means and standard deviations calculated. Qualitative variables were presented as frequencies and percentages. One-way ANOVA assessed quantitative variables, while chi-square assessed qualitative/categorical variables for the harmful effects of insecticidal modes. Significance was set at $p \leq 0.05$.

Results

Body weight

All animals meeting the inclusion criteria exhibited normal activity and alertness at the start of the experiment on day 1. The initial mean weights for groups I, II, and III were 28.21 ± 2.04g, 28.43 ± 2.24g, and 27.28 ± 1.54g, respectively.

Group Name	N	TBW_1		TBW_2		ΔTBW		P-Value
		Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	
I	14	28.214	2.0448	29.785	1.9286	1.571	3.3446	
II	14	28.428	2.2434	27.142	2.7416	-0.428	3.3675	
III	14	27.285	1.5406	20.642	4.0687	-6.642	3.2958	0.000

With 95% confidence interval a significant difference between initial and final weight was observed as the p value was less than 0.05 given in table 1.

During the experiment, noticeable behavioral changes were observed. Group III exhibited significant sluggishness and irregular eating habits, and some Group II animals displayed similar patterns. However, Group I maintained its initial level of activity. These observations aligned with the final body weights on day 61: Group I - 29.78 ± 1.93g, Group II - 27.14 ± 2.74g, and Group III - 20.64 ± 4.06g. The mean changes in body weight (ΔTBW = TBW2 - TBW1) were 1.57 ± 3.344g for Group I, -0.42 ± 3.36g for Group II, and -6.64 ± 3.29g for Group III.

Lungs weight variation

No significant difference of lung weight was observed between all three groups as the p value was more than 0.05. details of lungs weight are given figure 3

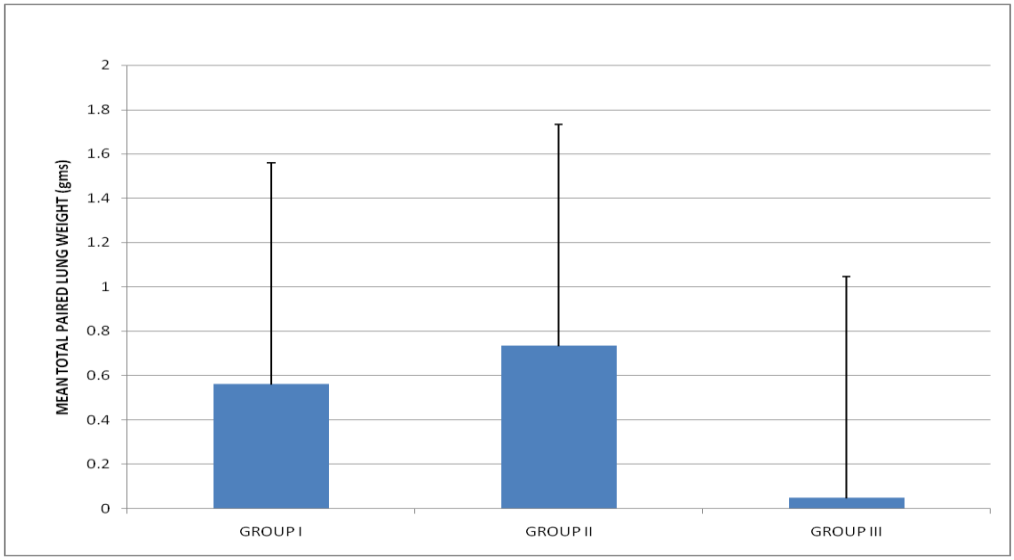


Figure 3 *Mean Lungs weight*

Lesion identification of lungs

The color of mouse lungs was compared to the shade card in Appendix VIII. All the lungs from Group I appeared fresh, exhibiting a smooth and shiny pink color (Fig. 2). In contrast, 78.6% of the lungs from Group II and 35.7% of the lungs from Group III retained their fresh pink color. Group III displayed the highest degree of color variations, including shades of grey, brown, and reddish hues, while Group II exhibited a lower percentage of lung discoloration. Significant difference in colour of the lungs among groups as p-value was less than 0.05.

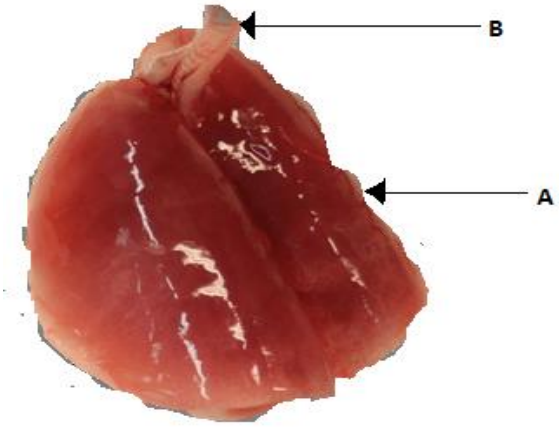


Figure 4 *Fresh pink shiny lungs (A) with no apparent gross abnormality and trachea (B)*

Figure 5 *Brown discoloration of lungs is seen*

Lung specimens obtained from the experimental groups were evaluated for their texture through palpation, using the index finger and thumb to assess softness, and by observing the release of air bubbles upon applying pressure. All lungs from Group I exhibited a soft and spongy texture, accounting for 100% of the samples. In contrast, 85.7% of the lungs from Group II and 78.6% from Group III were also found to be soft and spongy. Notably, 14.3% of the lungs from Group II and 21.4% from Group III were identified as having a harder and firmer texture. p-value ≤0.05 is considered statistically significant

Insignificant difference in percentages of lumps/nodules in dissected lungs from all groups shown in table

Group Name		Nodules or Lumps-Lungs		P-Value
		Absent	Present	
I	N	14	0	0.122
	%	100	0.0	
II	N	14	0	
	%	100	0.0	
III	N	12	2	
	%	85.7	14.3	

Tracheal examination revealed variations in the color of the airway lining, with Group I animals universally displaying a fresh pink appearance. In Group II, 78.6% exhibited fresh pink, 14.3% pale, and 7.1% reddish pink appearances. For Group III, only 14.3% showed fresh pink, while 78.6% displayed pale and 7.1% exhibited reddish tracheal linings. The presence of sore-like reddish patches resembling erosions was absent in all Group I animals, whereas 42.9% of Group III animals showed such patches, and only 7.1% of Group II animals displayed erosions. The tracheal mucosa was examined for texture, with a bumpy or rough granular texture noted in 35.7% of Group III animals and 7% of Group II animals, while all Group I mice had a smooth tracheal lining.

Normal and trachea with erosion is shown in figure left to right respectively

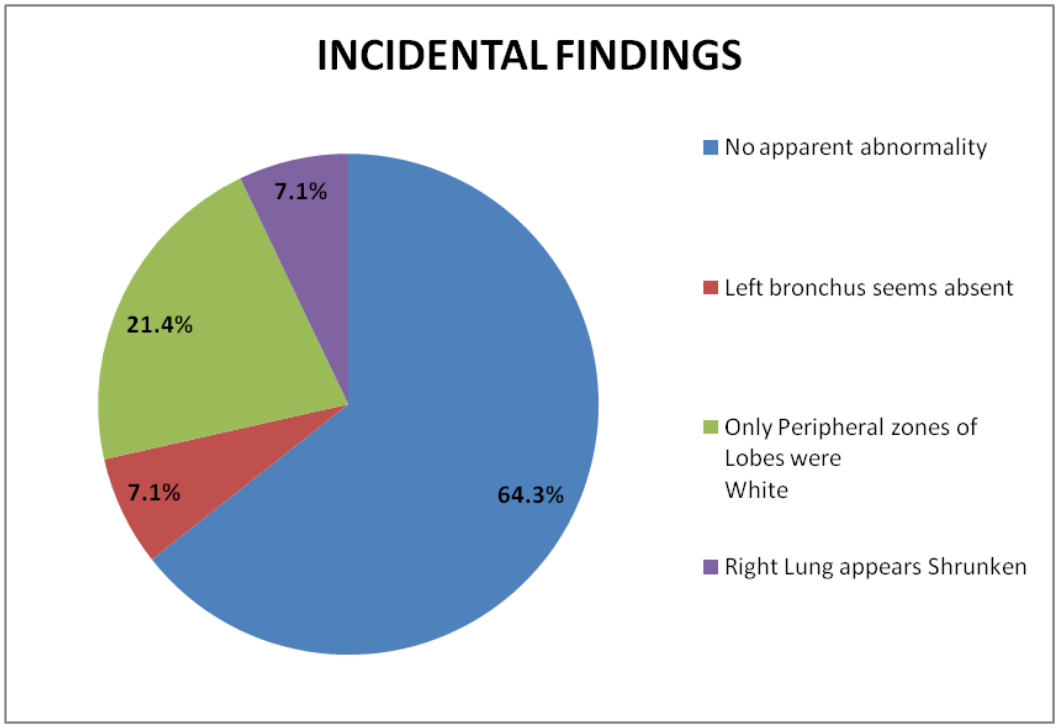
Tissues weight comparison

Average weight of tissues for group I, II and III was found to be 2.42 ± 0.41067, 2.70 ± 0.33 and 2.30 ± 0.77.

Group Name	N	Mean	Std. Deviation	P-Value
I	14	2.4279	0.41067	0.144
II	14	2.7079	0.33453	
III	14	2.3057	0.77254	

Incidental finding

Macroscopic examination of the pulmonary apparatus in Group I and Group II showed no gross abnormalities. However, in Group III, 21.4% of animals exhibited white peripheral zones in their lungs that appeared brown or pink. Additionally, 7.1% of Group III animals displayed an absence of the left bronchus, resulting in a direct attachment of the left lung to the lower end of the trachea via its hilum, while another 7.1% had a shrunken right lung



Discussion

The current study aimed to assess the comparative impact of two widely used mosquito repellent methods, mosquito coils, and liquid vaporizers, on respiratory airways. Monitoring general body weight and its fluctuations serves as a valuable indicator of overall health. To evaluate this, the initial and final body weights of the study subjects were recorded, and the difference between the two was calculated. The final body weight (TBW2) showed considerable variations among all three groups. In comparison to Group I (control), Group III (MCG) exhibited a significant decrease in weight, while Group II (LVG) displayed a minor decline. Allerthin vapours cause weight loss discussed in many studies (Abiodun et al., 2020a, YADAV et al., 2021) which was describe by this study as The mean changes in body weight (ΔTBW = TBW2 - TBW1) were 1.57 ± 3.344g for Group I, -0.42 ± 3.36g for Group II, and -6.64 ± 3.29g for Group III. The experiment, noticeable changes in behavior were also observed. Group III mice showed significant sluggishness and irregular dietary intake, with some animals in Group II also displaying similar patterns, these could also be the reasons of weight loss. Mice exposed to the noxious fumes emitted by mosquito coils displayed a decrease in both their respiratory parenchyma and stromal components. This observation aligns with a prior study that reported reduced organ weights, including lungs, kidneys, and liver, in experimental groups exposed to coil smoke for an extended duration of 8 weeks (Divakar et al., 2015). In the present investigation, notable color variations in lung specimens were observed among the test groups. Control animals exhibited a consistent and healthy fresh pink hue, with only three animals in Group II deviating from this norm. In contrast, Group III exhibited the highest degree of variation (ranging from 22% to 25%), displaying a spectrum of colors from reddish and brownish to greyish. Additionally, a whitish tinge was detected at the lobe edges in a subset of animals exposed to coil emissions, which might be attributed to the thickening of septa, interstitial, and stromal tissues. These findings were supported by a few previously published studies (Madhubabu and Yenugu, 2017) showed discoloration of pink lungs, (Garba et al., 2007) describe as the brownish color of lungs. Another study performed on inflammation of pulmonary viscera of Wistar rats exposed to allethrin coil showed gross examination of lumina of tracheobronchial structures, numerous erosions were observed (Abiodun et al., 2020b). Same symptoms were also observed in a study conducted by (Abiodun et al., 2020a) who used Wistar rats. Out of all the animals in Group III, only two exhibited the presence of nodular lumps upon visual inspection, while the remaining animals in both Group I and Group II did not display such a

characteristic. Remarkably, our statistical analysis yielded an insignificant p-value, indicating that the two-month exposure period was inadequate to induce any carcinomatous transformations in the subjects. While some studies, such as those by Shu-Chen et al. (2008) and Kyaw Myint Oo (2016), have strongly linked chronic exposure to mosquito coil smoke with lung cancer, it's important to note that their claims and inclinations towards this association may require a more prolonged duration of exposure to be adequately substantiated (Kyaw Myint, 2016) (Shu-Chen et al., 2008). The lung tissues in all three experimental groups exhibited a primarily soft and spongy consistency, with only minor variations linked to the observed changes in color. The statistical analysis, reflected in the non-significant p-value, aligns with the findings of a previous study that similarly reported no substantial alterations in lung tissue softness during a 90-day toxicity assessment involving allethrin. Same findings were observed by (Pauluhn and Mohr, 2006). The evaluation of allethrin, a common mosquito repellent component, on respiratory health revealed significant adverse effects. Mice exposed to allethrin through mosquito coil fumes displayed substantial changes in lung coloration, indicative of possible tissue damage. While no clear nodular lumps were observed within the two-month study period, these findings align with the notion that carcinogenic transformations may require a more extended duration of exposure. The overall consistency of lung tissues remained relatively unaltered, suggesting that allethrin may not affect lung softness in the short term.

Gap Analysis

This study, although shedding light on the potential harmful effects of allethrin, presents some gaps in our understanding. First, the relatively short duration of the study, two months, may not capture the long-term consequences of allethrin exposure, particularly concerning carcinogenicity. Further, the research primarily focuses on mice, and extrapolating these results directly to humans requires caution. Additional investigations should explore prolonged exposure durations, varying concentrations, and potential species-specific differences

Recommendation

This study highlights the need for further research to comprehensively understand the potential health risks associated with allethrin exposure. Extended, species-diverse studies, including humans, are crucial to discern long-term effects and carcinogenic potential. Evaluating varying concentration levels, identifying biomarkers for early detection, and enhancing public awareness about allethrin-related health risks is essential. Regulatory bodies should consider these findings when assessing the safety of allethrin-containing products and may impose appropriate safety measures and restrictions to protect public health

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