Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Acute toxicity of some expired insecticides on rats and their effects on some vital parameters and residues in the liver and kidney

Toxicidade aguda de alguns inseticidas vencidos em ratos e seus efeitos em alguns parâmetros vitais e resíduos no fígado e nos rins

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Abstract

Little information is available on the adverse effects of expired pesticides on the environment, so it is essential to characterize the risk of these chemicals to non-target organisms. Therefore, this work aims to estimate and compare the acute toxicity (LD_{50}) of unexpired and expired formulations of malathion, chlorpyrifos, and lambda-cyhalothrin in rats and to determine their residues in the liver and kidneys of treated rats. This is the first study to investigate the toxic effects of expired pesticides on rats. The acute toxicity of expired lambda-cyhalothrin was higher than that of non-expired rats, while the opposite was observed in rats treated with malathion and chlorpyrifos. All formulations tested caused clinical signs of toxicity in the treated rats. The data showed that some expired formulations significantly affected body weight and estimated vital signs compared to non-expired pesticides. The data showed that the highest residues were found in the liver and kidneys of rats treated with both malathion formulations, followed by chlorpyrifos; however, the lowest residues were found in rats treated with lambda-cyhalothrin, which can be referred to as LD50 values of the insecticides tested. The residues detected after the 10th dose gradually decreased at the end of the recovery period, and their losses ranged from 80.0 to 95.4% in the liver and from 92.3 to 99.99% (undetectable). The results show that the toxic effects of expired and non-expired formulations are different. This underlines the need to dispose of expired compounds carefully to prevent their discharge into the ecosystem.

Keywords: expired insecticides, acute toxicity, rats, vital parameters, residues.

Resumo

Poucas informações estão disponíveis sobre os efeitos adversos dos pesticidas vencidos no ambiente, por isso é essencial caracterizar o risco dessas substâncias químicas para organismos não alvo. Portanto, este trabalho visa estimar e comparar a toxicidade aguda (LD50) de formulações de malatião, clorpirifós e lambda-cialotrina em ratos e determinar seus resíduos no fígado e rins de ratos tratados. Este é o primeiro estudo a investigar os efeitos tóxicos dos pesticidas vencidos em ratos. A toxicidade aguda da lambda-cialotrina vencida foi maior do que a dos ratos não vencidos, enquanto o contrário foi observado em ratos tratados com malatião e clorpirifós. Todas as formulações testadas causaram sinais clínicos de toxicidade nos ratos tratados. Os dados mostraram que algumas formulações vencidas afetaram significativamente o peso corporal e os sinais vitais estimados em comparação com pesticidas não vencidos. Os dados mostraram que os resíduos mais elevados foram encontrados no fígado e nos rins dos ratos tratados com ambas as formulações de malatião, seguidos por clorpirifós; no entanto, os resíduos mais baixos foram detectados em ratos tratados com lambda-cialotrina, o que pode ser relacionado aos valores de LD50 dos inseticidas testados. Os resíduos detectados após a 10ª dose diminuíram gradualmente no final do período de recuperação, e suas perdas variaram de 80,0% a 95,4% no fígado e de 92,3% a 99,99% (indetectável). Os resultados mostram que os efeitos tóxicos das formulações vencidas e não vencidas são diferentes. Isso destaca a necessidade de eliminar cuidadosamente os compostos vencidos para evitar o seu descarte no ecossistema.

Palavras-chave: inseticidas vencidos, toxicidade aguda, ratos, parâmetros vitais, resíduos.

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Received: December 13, 2023 – Accepted: July 17, 2024

1. Introduction

Agriculture is the foundation of the global economy and accounts for 11.3% of the gross domestic product in Egypt, making it a vital sector of the national economy (Alston and Pardey, 2014; El-Ramady et al., 2013; Frascari et al., 2018). Pesticides have significantly increased agricultural production and food availability. Without pesticides, 78%, 54%, and 32% of fruit, vegetable, and cereal crops would be lost to pest infestation (Cai, 2008). Pesticides are seen as a quick fix for pest control to prevent crop damage but should only be used when all other control methods are unsuccessful. Among the other chemicals we are confronted with daily, pesticides occupy a unique position as they are intentionally introduced into the ecosystem to kill or injure life (Costa et al., 2008).

The widespread use of pesticides has led to problems such as ill-considered increases in production and the illegal trade in pesticides, leading to stockpiling. Expired pesticides as obsolete chemicals are a new challenge in the modern world. In recent decades, these pesticide stocks have accumulated in most developing and emerging countries (Hardiman, 2012). When a pesticide is expired, it can no longer be used and must be disposed of. The degradation of pesticides can cause the active ingredient to break down and/or physical and chemical changes to occur, resulting in an unacceptably high loss of biological effectiveness (Hajjar, 2015). Globally, it is estimated that over 500 thousand tons of banned, obsolete, damaged, and degraded products, unusable formulations and containers, unidentified products, and active ingredients or technical formulations of pesticides are consumed after the expiry date (Shalaby et al., 2018), destroying the ecosystem and human health (El-Bakry et al., 2021). Besides, Chlorpyrifos, Lambda-Cyhalothrin and malathion are widely used insecticides in agricultural and household pest control. Their extensive use increases the likelihood of their presence in expired products that might still be found in storage or inadvertently used. Besides these insecticides have well-documented toxicity profiles in their non-expired forms. Understanding how their toxicity might change after expiration is crucial for evaluating the risks associated with their use. Because the problem of obsolete pesticides is so severe, immediate action must be taken to eliminate current stocks and prevent further accumulation. High-temperature incineration is the method of hazardous waste disposal that is recommended as environmentally sound, although these facilities are not readily available in developing countries. Both the price of incineration and the logistical procedures required are expensive (Hajjar, 2015). Most developing countries have severe environmental and public health problems with obsolete pesticide stocks. When expired pesticides are present in the environment, they can pose new toxicological problems that differ from those of unexpired pesticides.

In some cases, it was observed that distributors passed such alleged expired compounds to farmers, which limited most farmers who knew little about the selection of appropriate pesticides (El-Bakry et al., 2021; Satyavani et al., 2012)). Unfortunately, farmers often do not know where they get the pesticides, thus unknowingly introducing hazardous chemicals into the environment (Kreisler and Heiss, 2008). Another study conducted in Ethiopia's Rift Valley found that 62% of farmers do not pay attention to the expiration date when purchasing pesticides, and 10% of farmers stored leftover pesticides and used them the following year even though they had already expired (Haylamicheal, and Dalvie, 2009). According to a study in Egypt, 46.7% of farmers did not check the expiry date of a pesticide before buying it, and 80% of leftover pesticides were stored and used the following season (Shalaby et al., 2022). These pesticides end up in water bodies via agricultural runoff (Rajput, 2012; El-Bakry et al., 2021). This contaminates the environment and exposes people to it.

Haematological parameters are the fastest and most accessible indicators for health status assessment, and they are also known for their importance in population genetics and clinical biochemistry (Ahmed et al., 2020). Most studies have shown that haematological indicators can detect disease or stress in animals (Bancroft and Gamble, 2008). In the literature, many authors have investigated the reproductive toxicity of specific doses of cypermethrin. Still, more information is available on hepatotoxicity, nephrotoxicity, haematological changes, and behavioral toxicity at the particular dose of the present study. In addition, the data and studies on the potential toxic effects are insufficient. Since large amounts of expired pesticides are present in our environment, it is necessary to characterize the risk of these chemicals (Satyavani et al., 2012). Therefore, the objectives of this study were: 1) to estimate and compare the median lethal dose (LD_{50}) of expired and unexpired formulations of the tested insecticides in albino rats; 2) to evaluate the effects of sub-lethal doses of the tested chemicals on some vital parameters; and 3) to estimate the residues of these insecticides in the liver and kidneys of the treated rats.

2. Materials and Methods

2.1. Insecticides

The following insecticide formulations were selected for the current study as they are widely used in Egypt. Chlorpyrifos (Chlorzan 48% EC) and lambda-cyhalothrin (Lamdathrin 5% EC) were purchased from a commercial manufacturer, Kafr El-Zayat Pesticides and Chemicals Company, Egypt. The malathion pesticide (Nasr-Lathion 57% EC) was supplied by El Nasr Company for Intermediate Chemicals, Egypt.

All the above products (non-expired formulations) were procured from the market within the prescribed shelf life (2 years). The same expired pesticides were purchased (the expiry date was between 12 and 18 months after the expiry date) and tested simultaneously.

2.2. Chemicals

Certified reference standard of chlorpyrifos, lambda-cyhalothrin, and malathion (98% purity), purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Trisodium citrate ($Na3C6H5O7$), disodium acetate ($CH3COONa$), anhydrous magnesium sulfate ($MgSO_4$), and primary secondary

amine (PSA) were purchased from Agilent Technologies, Egypt. All solvents used were HPLC grade and purchased from Scharlau, Spain. A Milli-Q water purification system (Millipore, USA) produced deionized water.

2.3. Animal model

Male albino rats (Rattus norvegicus) weighing 120±10 g were obtained from the Animal Breeding House of the National Research Center (NRC). The rats were kept in the laboratory at ± 2 °C and 48% relative humidity. The rats were acclimatized for one week before the start of the experiment. All rats were maintained by the guidelines and welfare of the NRC animal house approved by the NRC local ethics committee and handled according to the "Guide for the Care and Use of Laboratory Animals" (Od and Oer, 2011).

2.4. Biochemical assays

The sera were separated from clotted blood (non-heparinized ampoules) after centrifugation at 3000 rpm for 10 minutes and stored at -20 °C until analysis. Liver injury was determined by measuring the activity of esterase enzymes (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) and kidney injury by measuring urea and creatinine concentrations. These parameters were analyzed spectrophotometrically using kits purchased from Bio-Marieux (France). An Olympus Au 4oo analyzer was used for automated clinical chemistry. A blood picture was performed using the Coulter Counter T890 (Coulter Counter Harpenden, UK).

2.4.1. Determination of median lethal dose (LD_{50})

According to the Finney method for estimating the median lethal dose (LD50), exploratory tests were carried out on six groups of two rats each (Finney, 1971). Chlorpyrifos, cyhalothrin, and malathion (expired and unexpired formulations) were administered orally in five graded doses to six groups. The aim was to determine the lowest toxic dose. Doses of 800, 80, and 20 mg kg^{-1} body weight of malathion, chlorpyrifos, and lambda-cyhalothrin, respectively, were the first to induce signs of toxicity, multiplied by a constant factor (1.5) for each subsequent group of rats. The mortality of the rats was determined after 24 hours. The toxicity indices of the tested insecticides were estimated according to Sun's method (1950) (Sun, 1950).

2.4.2. Subacute toxicity

The animals were divided into seven groups of ten rats each to investigate the toxicity of the tested substances for biochemical markers. The first, second, third, fourth, fifth, and sixth groups received a dose of lambda-cyhalothrin (expired and unexpired) equivalent to one-tenth of the estimated median lethal dose (1/10 LD_{50}). The rats in the seventh group were used as controls. Every three days of the month, the toxins dissolved in corn oil were administered orally by gavage. To give the animals time to recover from the toxicity, the pesticides were discontinued for 15 days. All animals were examined daily for signs of pharmacological or toxicological effects.

2.4.3. Bleeding regimen

The rats bled after 24 hours for the 5th and 10th doses administered on days 15 and 30 and after the recovery period (15 days after the last dose). Under anesthesia, blood was collected from the retro-orbital plexus of the rats using heparinized and non-heparinized ampoules (Schalm et al., 1975).

2.4.4. Haematological effects

Blood in heparinized ampoules was tested for white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), and platelet (PLT) counts (Schalm et al., 1975).

2.5. Sampling and analytical methods

After the 10th dose and at the end of the recovery period, the labeled polyethylene was anesthetized; the liver and kidney were quickly separated, cleaned of foreign tissue, and stored in small labeled polyethylene bags at -20 ºC until analysis.

The method for determining the tested insecticides in liver and kidney samples was performed using the QuEChERS method, described previously but with some modifications (Lakshmanan et al., 2016). In brief, 0.5 g of the homogenized subsample (liver/kidney) was weighed into a 50-mL PTFE centrifuge tube, and 10 mL of acetonitrile containing 1% acetic acid was added and shaken for 1 min. Then 4 g MgSO4 and 1 g NaCl were added, shaken, and centrifuged at 5000 rpm for 5 minutes. A 5-mL aliquot of the supernatant was transferred to a 15-mL PTFE centrifuge tube containing 300 mg PSA and 1.8 g $MgSO_4$. A 0.22-micron PTFE filter (Millipore, Billerica, MA) was used to filter 2 mL of the supernatant, which was then transferred to a glass vial for HPLC-DAD analysis.

The insecticide samples tested were analyzed using an Agilent 1100 HPLC system equipped with a quaternary pump, an automatic injector, a thermostatic compartment for the column, and a photodiode array detector. The chromatographic column was a C18 Zorbax XDE (25 mm x 4.6 mm. 5 µm). The column was kept at room temperature. The flow rate of the mobile phase (acetonitrile/water = 50/50 v/v) was 0.8 ml/min, and the injection volume was 20 µl. Under these conditions, the retention times for chlorpyrifos, lamb-da--cyhalothrin, and malathion were 5.0, 7.52, and 9.22 minutes, respectively.

2.6. Analytical method validation

Following the SANTE/12682/2019 guideline, validation was carried out in which the following parameters were assessed: Linearity, the limit of quantification (LOQ), recovery, and precision (SANTE/12682/2019, European Union (2019)). This ensured that the analysis technique was suitable for the intended use. Linearity was evaluated in this study using matrix-matched calibration standards in the 0.01-10 mg/L range. The correlation coefficient (R^2) for the standard calibration curve of each target insecticide was significant (> 0.998). The accuracy of the analytical method was investigated by spiking liver and kidney samples from untreated rats with known amounts of chlorpyrifos, lambda-cyhalothrin, and malathion standards

at five concentrations ranging from 0.05 to 3.0 mg/kg. The mean recovery rate of the insecticides tested was over 80%, with an inter-day and intra-day precision (RSD) of 12%. This demonstrates the reproducibility and accuracy of the established method. Blank samples were prepared and analyzed as previously described to evaluate the reliability and precision of the process. The LOQ was determined to be the lowest level of fortification that met the acceptable criteria for precision and accuracy. The current study set the LOQ at 0.05 mg/kg for chlorpyrifos, lambda-cyhalothrin, and malathion.

2.7. Statistical analysis

Data were analyzed using analysis of variance (one-way classification ANOVA) followed by the least significant difference, LSD, at 5% (Costat Statistical Software, 1990).

3. Results and discussion

3.1. Biochemical assays

3.1.1. Determination of LD_{50} value

The data presented in Table 1 and Figure 1 show a difference in the acute toxicity of the expired and unexpired formulations of the tested compounds. Unexpired formulations of malathion and chlorpyrifos were more toxic than expired formulations. Their median lethal dose (LD_{50}) values were 1963.5 and 126.2 mg/kg, while the values for expired compounds were 2366.6 and 226.4 mg/kg, respectively. This could be due to the pesticide impurities that could alter the product's physical properties or increase the pesticide's toxicity (Ambrus et al., 2003). Similar results were obtained by Satyavani et al. (2012), who estimated the acute toxicity of 15 expired pesticides to fish and showed that the LC_{50} values (median lethal concentration) for expired pesticides were significantly lower than for non-expired pesticides (Satyavani et al., 2012). Rajput (2012) also reported that the LC_{50} values of expired pesticides in freshwater fish (Catlacatla) were lower than the corresponding values of non-expired products (Rajput, 2012).

In contrast, our results showed that the expired cyhalothrin was more toxic than the non-expired one; its LD50 values were 39.3 and 55.2 mg/kg body weight in treated rats. The increased toxicity of expired pesticide formulations is attributed to active ingredients' by-products or impurities in the pesticide formulation that may be more hazardous than the parent compounds. Compared to non-expired insecticides, the toxicity of expired insecticides may be lower because their metabolites are less degraded than the parent compounds (Satyavani et al., 2012).

3.1.2. Histopathological Changes

3.1.2.1. Clinical signs

All treated and untreated animals were observed daily for signs of pharmacological or toxicological effects. Some clinical signs of toxicity occurred in the treated rats (Table 2), including salivation, diarrhea, closed eyes, increased activity, lacrimation, and aggressiveness. In addition, two animals died in rats treated with unexpired chlorpyrifos (after the 8th and 9th doses) and in rats treated with expired cyhalothrin (after the 8th and 10th doses). At the same time, no mortality was observed in the other treatments. The same results were obtained by Kumar (2018), who reported that physical parameters were impaired in deltamethrin-treated rats; after the first dose, the rats became hyperactive, probably manifested by sneezing, trembling, moaning, and excessive salivation that lasted for half an hour. Later, the animals appeared lethargic; loss of appetite was accompanied by diarrhea and vomiting (Kumar, 2018). Ahmed et al. (2020) also reported no clinical signs in rats treated with 0.5 and 1.0 mg/kg body weight of the fungicide cymoxanil for 21 days. While 2.0 mg/kg caused drowsiness and incoordination,

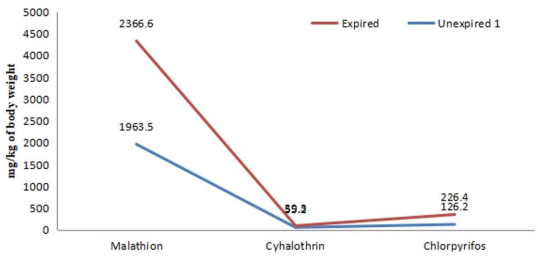

Figure 1. Median lethal dose of expired and unexpired formulations of tested insectides.

Table 1. Toxicity index for unexpired and expired tested pesticides on white albino rats.

Pesticides		LD_{50}	LD_{95}	Slope	Toxicity index (%)	
					LD_{50}	LD_{95}
Malathion	Unexpired	1963.5	14246.3	1.83	100	100
	Expired	2366.6	15470.0	2.11	83.0	92.1
Chlorpyrifos	Unexpired	126.2	759.8	2.11	100	100
	Expired	226.4	2401.2	1.6	55.7	31.6
Lambda-Cyhalothrin	Unexpired	55.2	1461.4	1.66	71.1	21.3
	Expired	39.3	311.5	1.83	100	100

decreased body weight, and reduced feed intake, no deaths were observed in treated animals during the study period (Ahmed et al., 2020).

3.1.2.2. Effects on body weight

The results in Table 3 and Figure 2 show no significant change in the body weight gain of the treated rats after the 5th and 10th doses. A significant decrease was observed in rats treated with expired malathion and chlorpyrifos after the 5th dose compared to the non-expired formulations of the two insecticides. After the recovery period (15 days after the last dose), an increase in body weight was observed in all animals. However, there was a significant increase in untreated rats compared to other treatments. Kumar (2018) observed decreased body weight in rats after treatment with deltamethrin compared to control (Kumar, 2018).

3.1.2.3. Clinical signs

Blood is a sensitive indicator of physiological changes in an animal reacting to environmental pollutants. It is well known that toxic stress of any kind leads to conspicuous and significant changes in hematological measurements (Shalaby et al., 2022). The data obtained showed that malathion formulations caused a decrease in hemoglobin concentration, while cyhalothrin caused a considerable increase after the 5th dose compared to untreated rats (Table 4). After the 10th dose and the recovery period, hemoglobin levels were elevated in all treated rats; expired chlorpyrifos and non-expired cyhalothrin caused the most significant increase (mean concentrations were 13.1 and 13.6 mg/dl; 11 and 15.3 above the control value, respectively). Red blood cell counts also increased significantly after the 5th dose in rats treated with expired chlorpyrifos and unexpired cyhalothrin, and a significant increase in red blood cell counts was observed after the 10th dose and the recovery period in all treatments. The most significant increase was observed in rats treated with expired chlorpyrifos and unexpired lambda-cyhalothrin (mean values were 6.6 and 6.64 × 106; 41.9 and 42.8 above average, respectively).

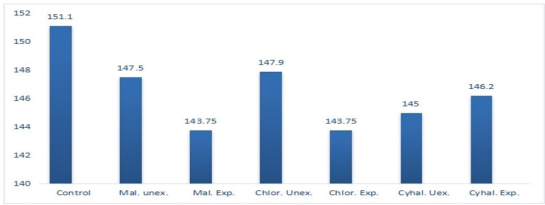

Figure 2. The average body weight of treated rats.

Table 2. Toxic symptoms which noticed in treated animals during the experiment period.

Pesticides		Signs of toxicity	Animal mortality
Control		Nil	No mortality
Malathion	Unexpired	Salivation and diarrhoea.	No mortality
	Expired	Diarrhea and increased activity	No mortality
Chlorpyrifos	Unexpired	Salivation, diarrhoea and closed eyes	Two animals were dying at the 8th and 9th dose
	Expired	Increased activity and lacrimation	No mortality
Lambda-Cyhalothrin	Unexpired	Diarrhea, lacrimation and salivation	No mortality
	Expired	Increased activity, aggressiveness, diarrhoea and closed eyes	Two rats were dying at the 8th and 10th dose

Table 3. Effect of unexpired and expired formulations of tested pesticides on body weight (g) of treated rats.

Pesticides		Pre-treatment	5th dose	10th dose	After recovery period
Control		114.33^{b}	146.67^{abc}	160.0^{a}	183.33^{a}
Malathion	Unexpired	116.67^{ab}	153.33^{a}	156.67^{a}	163.33^{cd}
	Expired	118.33^{ab}	136.67^{c}	156.67^{a}	163.33^{cd}
Chlorpyrifos	Unexpired	115.0^{b}	150.0^{ab}	156.67^{a}	170.0^{bc}
	Expired	120.0^{b}	138.33^{c}	160.0^{a}	156.67^{d}
Lambda-Cyhalothrin	Unexpired	123.33^{a}	140.0^{bc}	156.67^{a}	160.0^{d}
	Expired	120.0^{ab}	138.33^{c}	153.33^{a}	173.33^{b}
LSD 5%		7.89	11.42	12.48	9.37

The letters indicate significant differences between treatments and the control; each figure between brackets represents the content percentage as a check.

Table 4. Effect of unexpired and expired formulations of tested pesticides on red blood cell counts and hemoglobin value of treated albino rats.

Treatments		Haemoglobin value (mg/dl)				Red blood cells (10^3)			
		5th dose	10th dose	Recovery	Mean	5th dose	10th dose	Recovery	Mean
Control		11.2^{b}	12.7^{bc}	11.6^{c}	11.8	4.84^{bc}	4.81^{d}	4.3^{d}	4.65
Malathion	Unexpired	10.6^{bc}	12.8^{bc}	14.0^{a}	12.47 (±5.7)	4.94^{bc}	6.35^{bc}	7.39^{a}	6.23 (±34.0)
	Expired	9.7^{cd}	13.1^{ab}	13.9^{ab}	12.23 (±3.6)	5.05^{bc}	6.29^{bc}	6.84^{b}	6.06 (±30.3)
Chlorpyrifos	Unexpired	11.3^{bc}	13.5^{ab}	13.6^{ab}	12.8 (±8.5)	5.38^{b}	6.61^{b}	7.05^{ab}	6.35 (±36.6)
	Expired	11.3^{bc}	13.7^{ab}	14.3^{a}	13.1 (±11.0)	5.71^{ab}	6.7^{b}	7.38^{a}	6.6 (±41.9)
Lambda-Cyhalothrin	Unexpired	12.7^{a}	14.1^{a}	14.1^{a}	13.6 (±15.3)	5.95^{a}	6.85^{ab}	7.13^{a}	6.64 (±42.8)
	Expired	12.1^{a}	13.2^{ab}	13.2^{bc}	12.8 (±8.5)	6.12^{a}	6.54^{bc}	6.82^{b}	6.5 (±39.8)
LSD 5%		1.0	1.1	1.2		0.6	0.4	0.5	

The letters indicate significant differences between treatments and the control; each figure between brackets represents the content percentage as a check.

Table 5. Effect of unexpired and expired formulations of tested pesticides on white blood cells and Platelets counts of treated albino rats.

Treatments		Platelets count (10^3)				White Blood Cells (10^3)			
		5th dose	10th dose	Recovery	Mean	5th dose	10th dose	Recovery	Mean
Control		452^{d}	430^{cd}	417^{c}	433.0	4.9^{d}	5.5^{d}	6.4^{d}	5.6
Malathion	Unexpired	480^{d}	438^{cd}	677^{b}	531.7 (±22.8)	8.6^{bc}	7.6^{bcd}	15.2^{a}	10.47 (±86.9)
	Expired	413^{d}	550^{c}	818^{a}	593.7 (±37.1)	3.7^{de}	11.7^{a}	13.2^{abc}	9.53 (±70.2)
Chlorpyrifos	Unexpired	790^{b}	540^{c}	905^{a}	745.0 (±72.1)	13.3^{a}	11.5^{a}	12.6^{abc}	12.47 (±122.7)
	Expired	908^{a}	649^{bc}	774^{ab}	777.0 (±79.4)	6.3^{cd}	9.1^{abc}	11.7^{bc}	9.03 (±61.3)
Lambda-Cyhalothrin	Unexpired	653^{c}	544^{c}	659^{b}	618.7 (±42.9)	7.8^{c}	10.7^{a}	11.0^{c}	9.83 (±75.5)
	Expired	667^{c}	774^{a}	913^{a}	784.7 (±81.2)	5.4^{cd}	9.2^{abc}	10.8^{c}	8.47 (±51.3)
LSD 5%		115	120	140		2.7	2.8	2.9	

The letters indicate significant differences between treatments and the control; each figure between brackets represents the content percentage as a check.

3.1.2.4. Haematological effects on treated rats

Thrombocytes play an essential role in homeostasis and coagulation processes in the body and originate from the bone marrow. Thrombocytopenia is defined as an insufficient number of platelets in the circulating blood (Shalby, 2006). This is the most common cause of abnormal bleeding in living organisms. The data in Table 5 show that both chlorpyrifos and lambda-cyhalothrin caused a significant increase in platelet counts in the treated rats after the 5th dose. This increase continued after the 10th dose; after the recovery phase, all insecticides tested caused a more significant increase in platelet counts than in untreated rats. The highest increase was observed in rats poisoned with chlorpyrifos and cyhalothrin (777.0 and 784.7 × 10^3; 79.4 and 81.2 above the control value, respectively). A similar effect was observed in the white blood cell (WBC) count. After the 5th dose, there was a significant increase in rats treated with 1/10 LD_{50} of the unexpired formulations of the insecticides tested. In addition, all formulations tested caused a significant increase in WBCs after the 10th dose, except in rats treated with unexpired malathion. After the recovery period, these effects increased compared to the untreated rats. The most significant increase was observed in rats treated with unexpired chlorpyrifos (the mean value was 12.47 × 10^3, i.e., 122.7% higher than in the untreated rats). The sharp increase in the white blood cell count may be due to the inflammatory response induced as a defense mechanism. In addition, the tested compounds may influence the white blood cell count due to the stress-inducing effect of these insecticides on the reticuloendothelial system (Rettie et al., 1986; Shalby, 2006).

3.1.2.5. Histopathological Changes in the liver

Animals inhale, ingest, and absorb many chemicals in their living environment, which can cause harmful effects

and tissue damage via numerous biochemical mechanisms. The liver is the main organ for the biotransformation of xenobiotic chemicals, so it is susceptible (Shalby, 2006; Shalaby et al., 2022). The data in Table 6 showed no significant change in (ALT) enzyme activity compared with untreated rats after the 5th dose, except in the rats treated with expired chlorpyrifos, which showed a considerable increase. After the 10th dose, there was a significant increase in this enzyme activity in all formulations tested. This increase gradually decreased during recovery and reached normal levels in rats poisoned with lambda-cyhalothrin formulations and unexpired chlorpyrifos. The highest enzyme activity was observed in rats treated with expired chlorpyrifos (their mean value was higher than 49.0% of untreated rats). In the same table, the data showed a significant decrease in alanine aminotransferase (AST) activity in rats treated with cyhalothrin formulations and expired chlorpyrifos compared to untreated rats after the 5th dose.

In contrast, all insecticides tested caused a significant increase in this enzyme activity after the 10th dose. The same trend was observed during the period of recovery; enzyme activity gradually decreased but did not reach normal levels, except for unexpired cyhalothrin. Aminotransferase enzymes transfer the amino group of alanine and aspartate to alpha-ketoglutaric acid and form glutamic acid and pyruvic acid. The activity of these enzymes is measured in the livers of the treated animals. The deviation of the activities of these enzymes from average values indicates biochemical impairment and damage to tissues and cell functions, as they are involved in the detoxification process, metabolism, and the biosynthesis of active macromolecules for various vital functions (Shalby, 2006; Shalaby and El-Mageed, 2010).

3.1.2.6. Histopathological Changes in the Kidney

The sharp increase in creatinine and urea concentrations may be due to the role of insecticides in glomerular filtration, which subsequently increases serum creatinine uremia. This finding suggests that these compounds may induce renal damage or toxicity and lead to renal failure. The data in Table 7 show that after the 5th dose, there were

Table 6. Effect of unexpired and expired formulations of tested pesticides on liver functions of albino rats.

Treatments		ALT (µ/ml)				AST (µ/ml)			
		5th dose	10th dose	Recovery period	Mean	5th dose	10th dose	Recovery period	Mean
Control		102.1^{bc}	104.0^{c}	106.3^{c}	104.1	36.2^{a}	34.6^{d}	30.6^{c}	33.8
Malathion	Unexpired	107.0^{b}	150.9^{b}	121.4^{b}	126.43 (±21. 4)	38.8^{a}	51.5^{b}	50.0^{a}	46.8 (±38.4)
	Expired	91.7^{c}	152.5^{b}	146.7^{a}	130.3 (±25.1)	30.8^{b}	45.0^{c}	42.8^{b}	39.5 (±16.8)
Chlorpyrifos	Unexpired	107.9^{c}	205.7^{a}	114.2^{bc}	142.8 (±37.2)	37.9^{a}	66.7^{a}	40.7^{b}	48.4 (±43.2)
	Expired	124.1^{a}	198.9^{a}	142.3^{a}	155.1 (±49.0)	40.0^{a}	47.9^{b}	38.5^{b}	42.1 (±24.6)
Lambda-Cyhalothrin	Unexpired	91.9^{c}	153.1^{b}	109.5^{bc}	118.2 (±13.5)	32.0^{b}	46.7^{b}	30.0^{c}	36.2 (±7.1)
	Expired	103.3^{bc}	143.3^{b}	103.8^{c}	116.8 (±12.2)	28.5^{b}	40.8^{c}	40.7^{b}	36.7 (±8.6)
LSD 5%		11.4	14.6	12.7		4.1	5.2	4.6	

The letters indicate significant differences between treatments and the control; each figure between brackets represents the content percentage as a check.

Table 7. Effect of unexpired and expired formulations of tested pesticides on kidney functions of albino rats.

Treatments		Creatinine				Urea			
		5th dose	10th dose	Recovery Period	Mean	5th dose	10th dose	Recovery period	Mean
Control		0.56^{ab}	0.50^{b}	0.53^{c}	0.53	36.5^{d}	32.1^{d}	38.5^{f}	35.7
Malathion	Unexpired	0.46^{b}	0.64^{a}	0.68^{b}	0.59 (+11.3)	44.1^{c}	53.2^{a}	50.1^{de}	49.1 (+37.5)
	Expired	0.6^{a}	0.66^{a}	0.73^{ab}	0.66 (+24.5)	50.4^{ab}	49.0^{ab}	71.0^{a}	56.8 (+59.1)
Chlorpyrifos	Unexpired	0.66^{a}	0.69^{a}	0.75^{ab}	0.7 (+32.1)	46.9^{b}	46.3^{bc}	55.4^{c}	49.5 (+38.6)
	Expired	0.62^{a}	0.69^{a}	0.70^{ab}	0.67 (+26.4)	51.6^{a}	41.2^{c}	47.8^{e}	46.9 (+31.4)
Lambda-Cyhalothrin	Unexpired	0.67^{a}	0.72^{a}	0.75 ^{ab}	0.71 (+33.9)	43.3^{bc}	52.8^{a}	53.7^{cd}	49.9 (+39.8)
	Expired	0.62	0.7^{a}	0.80a	0.71 (+33.9)	45.8 ^{b}	48.5^{ab}	62.3^{b}	52.2 (+46.2)
LSD 5%		0.11	0.08	0.1		4.6	6.17	4.2	

The letters indicate significant differences between treatments and the control; each figure between brackets represents the content percentage as a check.

Table 8. Residue of tested pesticide amounts (mg/kg) in the liver and kidney of treated rats after the 10th dose and recovery period.

Treatments		Residues (mg/kg)					
		Liver			Kidney		
		After 10th dose	After recovery period	% Loss	After 10th dose	After recovery period	% Loss
Malathion	Unexpired	5.89	0.32	94.6	1.87	0.02	98.9
	Expired	6.8	0.26	91.1	2.25	0.13	94.2
Chlorpyrifos	Unexpired	0.38	0.023	93.9	0.13	0.01	92.3
	Expired	0.68	0.031	95.4	0.22	0.01	95.4
Lambda-Cyhalothrin	Unexpired	0.2	0.04	80.0	0.067	ND	> 99.9
	Expired	0.17	0.01	94.1	0.06	ND	> 99.9

ND = non-detected.

no significant changes in blood creatinine concentrations in all treated rats compared to untreated rats. After the 10th dose, all compounds tested caused a significant increase in creatinine concentration; this increase continued during the recovery phase. The highest increase was observed with lambda-cyhalothrin formulations (33.9% above average). A similar trend was observed for urea concentration; all insecticides tested caused a significant increase in blood urea concentration after the 5th and 10th dose and reached the highest values during the recovery phase. The highest mean value was observed in rats treated with expired malathion and cyhalothrin (59.1 and 46.2% above the control value). In general, the increase in creatinine concentration is considered a biomarker of renal damage and can be attributed to liver function, and the increase in urea may be due to a disturbance in protein metabolism (Ahmed et al., 2020).

3.1.3. Pesticide residues in the liver and kidney

The liver is the primary site for detoxification or biotransformation of xenobiotics, and it may be the final target site of the pesticide (Heikal et al., 2012). The data presented in Table 8 show that the detected pesticide residues were higher in the liver tissue than in the kidneys of the treated rats. In addition, higher residues were detected in the rats treated with expired malathion and chlorpyrifos than in those not treated with expiry compounds, while the opposite was observed in the rats treated with lambda-cyhalothrin. At the same time, the residues detected after the 10th dose decreased at the end of the recovery period, with losses in the liver ranging from 80.0 to 95.4%, while in the kidneys, they ranged from 92.3 to > 99.99% (undetected). The data show that the highest residues were found in rats treated with both malathion formulations, followed by chlorpyrifos, while the lowest residues were detected in the organs of rats treated with lambda-cyhalothrin; this can be attributed to the LD_{50} values of the insecticides tested. In comparison, the LD_{50}s values of unexpired and expired malathion were 1963.5 and 2388.6 mg/kg, while the corresponding values of chlorpyrifos and lambda-cyhalothrin were 126.2, 226.4 and 55.2, 39.3 mg/kg, respectively. The data of Tanvir et al. (2016) showed that about 6.18% of chlorpyrifos was distributed in body tissues, and the highest residues (3.80%) were detected in fatty tissue, which was also found in the liver, brain, kidney, and ovaries (0.29, 0.22, 0.10 and 0.03%, respectively) (Tanvir et al., 2016). Kumar et al. (2011) also reported that 9.05% of buffalo liver samples from four slaughterhouses contained chlorpyrifos residues (23 of 254 samples); only 0.78% of the samples analyzed exceeded the Codex maximum residue level (Kumar et al., 2011).

4. Conclusion

Expired pesticides are a significant problem for the environment and public health. In most developing countries, there is a lack of efficient management and accessible disposal strategies. Expired pesticides can cause other environmental chemical and toxicological problems that have nothing to do with the original substances. According to the experiment results, specific pesticide formulations became even more harmful after their shelf life expired. Therefore, more efforts need to be made to find new approaches for the disposal of obsolete pesticides. We, therefore, propose that strict legislation be introduced to ensure that expired pesticides never re-enter the environment and are properly destroyed or disposed of without harming the soil ecology.

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