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ACUTE TOXICITY STUDY OF D-TRANS ALLETHRIN AND D-PHENOTHRIN TO ZEBRAFISH, *DANIO RERIO* AND ITS HUMAN RELEVANCE

Abdul Alif Abd Hamid [1]*, Muhammad Lokman Md Isa [2], Lee Siew Pien [3], Nik Fakhuruddin Nik Hassan [4], Hussin Muhammad [5] and Harmili [6]

[1] PhD, Department of Critical Care Nursing, Kulliyyah of Nursing, International Islamic University Malaysia, Indera Mahkota Campus, 25200 Kuantan, Pahang, Malaysia.
*Corresponding Author Email: alifhamid@iium.edu.my
[2] PhD, Institute of Planetary Survival for Sustainable Well-being (PLANETIIUM), Level 2, International Islamic University Malaysia, Jalan Hospital, 25100 Kuantan, Pahang, Malaysia.
[3] PhD, Department of Special Care Nursing, Kulliyyah of Nursing, International Islamic University Malaysia, Indera Mahkota Campus, 25200 Kuantan, Pahang, Malaysia.
[4] PhD, School of Health Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia.
[5] PhD, Herbal Medicine Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia, Level 5, Block C7, No. 1, Jalan Setia Murni U13/52, Seksyen U13, Setia Alam, 40170 Shah Alam Selangor, Malaysia.
[6] Nursing Study Program, STIKES Griya Husada Sumbawa, West Nusa Tenggara, Indonesia.

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Abstract

In Malaysia, insecticide spray is widely used in a household environment, and most consumers are not aware of its exposure effects. As evidence by reported insecticide intoxication cases involving hospitalisation and fatality cases suggested insufficient studies on insecticide usage and its exposure effects. Commonly insecticide constitutes of type 1 pyrethroids with allethrin and d-phenothrin featured prominently in most insecticide products. Hence, the acute toxicity study of allethrin and d-phenothrin mixture which represents the insecticide consumer product was conducted to provide data on insecticide consumer product toxicological information. Adult zebrafish were randomly selected and exposed to the insecticide mixture. The clinical sign of toxicosis and mortality rate of the zebrafish were observed in 96 h period. The toxicosis characteristics of the zebrafish during the study were documented and interpreted using the OECD TG203 and the mortalities were computed and analysed using statistical package SPSS version 28. Based on the LC_{50} obtained the HED and MTD were calculated. The insecticide-exposed zebrafish exhibits changes in their locomotion and respiration. The LC_{50} values for d-trans allethrin and d-phenothrin were 0.80 and 0.51 µg/L with HED were 0.003 and 0.002 µg /kg and the MTD were 0.18 and 0.12 µg /kg respectively. The finding indicates insecticide toxicity effects were observed in the zebrafish model. Considering the toxicity effects, levels, insecticide required and extrapolated MTD data, there is a legitimate risk exposure for humans when these substances were extensively used daily in the home environment.

Keywords: Acute Toxicity Test, Home Environment, İnsecticide Exposure, İnsecticide Consumer Product, İnsecticide Toxicity Test.

INTRODUCTION

In Malaysia, insecticide spray is widely used in a household environment, and most consumers are not aware of its exposure effects (1). The frequent exposure to the household through inhalation is a concern. An insecticide exposure case was reported in Batu Gajah, Perak, recently, with more than 100 victims needed medical treatment, 37 victims hospitalised, 4 victims in critical condition, and a fatality (2). There were 47% accidental exposure hospital admission recorded in Malaysia with fatality occurrence for insecticide or pesticide exposure cases (3).

The most common insecticide chemical used is a pyrethroid that may exert toxicity effects through its mechanism that react and alter the sodium and chloride channels of human cells, especially at the nerve ending cell (4). The chemicals modify the sodium channel physiology, subsequently increasing the sodium permeability of the cell membrane and triggers multiple action potential (5). Thus, the repeated action potential may result in hyperexcitability of cells that leads to tremor, paraesthesia, elevated body temperature, coma, and even death (ATSDR, 6).

The zebrafish (*Danio rerio*) model is the latest and popular trend to identify the toxicity levels of household insecticide due to its short life cycle and low cost (7). The primary benefit of using zebrafish is that insecticide can be added to the zebrafish tank according to the desired concentration, as its toxicity kinetics is comparable to other models with less labour intensity and use of chemicals (8). Besides, insecticides are usually water-based that can be used in zebrafish toxicity study.

This study was performed to determine the insecticide toxicity levels to provide data on insecticide consumer product toxicological information.

MATERIALS AND METHODS

Study design

Samples of mature 6-months-old wild-type zebrafish (*Danio rerio*) with 3.79 ± 0.24 cm and 1.87 ± 0.09 g in length and weight were selected from a local laboratory fish breeder (Danio Assay Laboratories, Sdn. Bhd., UPM, Selangor, Malaysia). The fish were acclimatised for two weeks. The fish were randomly placed in each fish tank with a density of 10 fish per tank (9).

This study used a mixture of two chemicals, with a concentration of d-trans allethrin and d-phenothrin at 0.11% and 0.07% (w/w) available in the form of a commercial insecticide consumer product purchased from a local market. The mixture was obtained from the aerosol spray can by spraying it through the nozzle into a sealed canister and used as the stock solution for the study.

Based on the preliminary study, the toxicity test for the insecticide mixture was determined at 24, 48, 72, and 96 h upon exposure levels with 5 different concentrations of insecticide mixture designed with a factor of 2 (0.20, 0.40, 0.80, 1.60, and 3.20 µg/L for d-trans allethrin and 0.13, 0.25, 5.10, 1.00, 2.00 µg/L for d-phenothrin) diluted in water containing 0.1% acetone, according to the Organisation for Economic Co-operation and Development (OECD) guideline (10).

The zebrafish were separated into a control group (group 1) and insecticide mixture-treated group (group 2-6) with N = 10 for each experiment (11). The control group used tap water containing 0.1% acetone, with an equivalent volume of the stock solution. The stock solution was changed every 24 hours (12). The fish was considered dead if the gill movement is diminished and unresponsive to gentle poking and removed using a plastic spoon to avoid contamination. The toxicity study was conducted in triplicate, and the LC_{50} of the insecticide mixture was determined based on observed zebrafish mortality.

Measure and analysis

The clinical sign of toxicosis characteristics of the zebrafish during the study were identified, assessed, documented and interpreted using the OECD Test Guideline 203 (TG203) (10).

The mortalities zebrafish control and insecticide mixture treated groups at different concentration and exposure periods were recorded, computed, and analysed using statistical package SPSS version 28 (IBM, Chicago, Illinois, USA) (13). The chi-square values, LC_{50} values, correlation regression (R^2), upper and lower confidence limits (UCL and LCL), and the slope were determined.

The determined LC_{50} values of each d-trans allethrin and d-phenothrin were extrapolated to the human dose (14).The human equivalent dose (HED) formula presented below was employed as indicated by the Food and Drug Administration (FDA) for extrapolation purposes:

HED (mg/kg) = Animal LOAEL mg/kg × (Animal Weight kg/ Human Weight kg) (1–0.67)

The animal lowest observed adverse effect level (LOAEL) was determined by calculating the LC_{50}, the animal weight was determined earlier as 1.87 g, and human weight was standardised at 60 kg. The HED value was then divided with the safety factor of 10 to determine the acute exposure values for humans per kilogram (14).

The Maximum Tolerated Dose (MTD) was calculated by multiplying the HED by 60 (60 kg of adult human body weight), as per the formula below:

MTD (mg/day) = HED mg/kg × 60 kg

The acute dosage estimation calculation was calculated using the drug preparation formula, as presented:

Dosage required (mL) = (MTD mg/day ÷ Stock drug in a Solution mg) × 1000 mL/1

Hence, the dose required was calculated by dividing MTD with the stock drug representing the volume of insecticide from the stock solution (15). The researcher opted to covert the mass unit from mg to µg to produce systematic scientific readable data for the study.

Ethical consideration

The study was performed in line with the recognised guideline of humane animal care and permitted by the International Islamic University Malaysia Animal Care and Use Committee (IACUC-2020-03), under the strict regulation legislation of Animal Welfare Act 2105 (AWA 2015) (16).

RESULTS

Determination of zebrafish insecticide toxicity levels

The zebrafish exposed to insecticide exhibits behavioural changes in terms of their locomotion and respiration. All characteristics mentioned were identified and assessed using the OECD Test Guideline 230 (TG230) based on the suitability of the study and presented in Figures 1, 2, and Table I (OECD, 10).

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Figure I: The intoxicated zebrafish rolls vertically prior to death

Figure 2: The intoxicated zebrafish's body surface darkened

Table I: Clinical signs observed in zebrafish

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Loss of Equilibrium	-	+	+	++	+++	+++
Loss of Ventilation	-	+	+	++	+++	+++
Abnormal Skin Pigmentation	-	+	+	++	+++	+++

+: The criteria have been met expressed (+ low impact, ++ visible and distinct underwater and +++ rapid identification in the water)

-: does not meet the criteria

The data showed that group 1 did not express any clinical criteria, while groups 2 and 3 showed minimal clinical signs, with a slight leaning of the fish movement in its attempt to correct the equilibrium. This was accompanied by minor darkening of the skin and opercular movement.

The clinical signs for group 4 were highly visible even as the fish was underwater, with swimming movement characteristic of leaning towards the heavy side, increased ocular movement, and tan or brownish colour of skin. Subsequently, the researcher rapidly identified the criteria in groups 5 and 6, whereby the fish rolled upside down, with darkened skin colour and robust ocular movement.

At the end of 96 h of insecticide exposure, the mortality rates of the zebrafish at specific time intervals at 24, 48, 72, and 96 h of insecticide exposure were observed, and a graph was plotted (Figure 3).

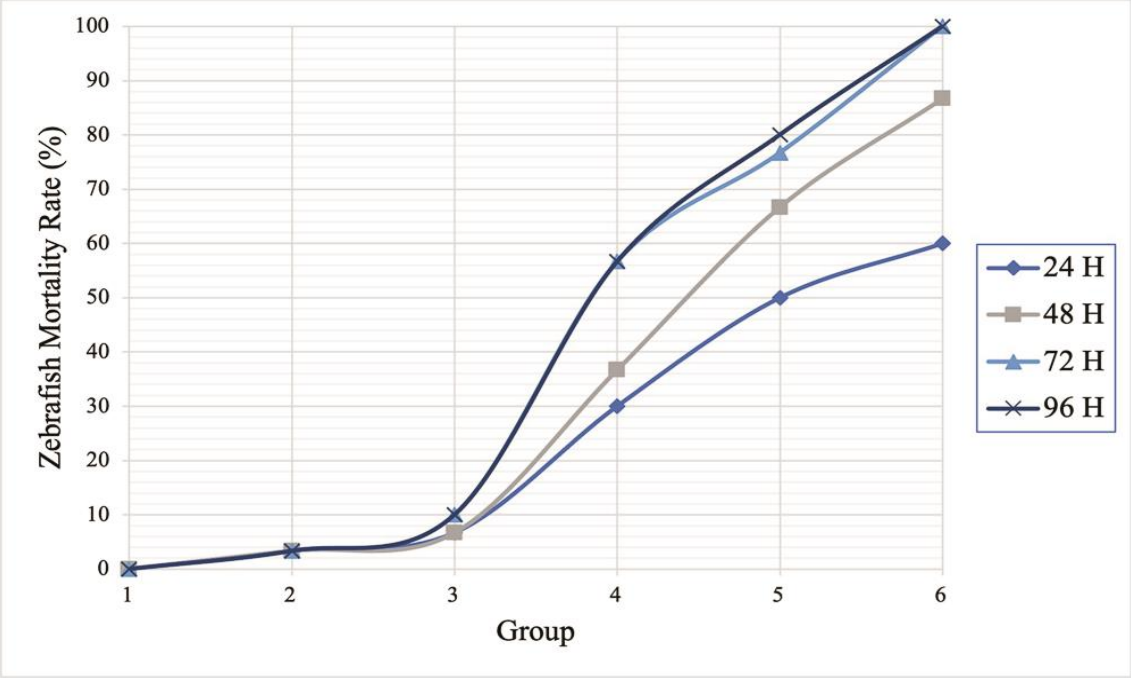

Figure 3: Insecticide dosage dependant toxicity in zebrafish mortality rate at 24, 48, 72, and 96 h

The mortality rate for each time interval is presented according to the six concentration groups. The mortality rates of 0%, 3.3%, 6.7%, 30%, 50%, and 60% are reported for 24 h insecticide exposure period. Meanwhile, the mortality rate for 48 h time interval are 0%, 3.3%, 6.7%, 30%, 66.7%, and 86.7%. The mortality rates increase for the 72-h exposure period, yielding 0%, 3.3%, 10%, 56.7%, 76.7%, and 100%. Finally, for the 96-h period, the mortality rates are 0%, 3.3%, 10%, 56.7%, 80.0%, and 100%. Overall, the control exhibits 0% mortality rate across all exposure time. Based on the initial data of mortality rate across the exposure period, the LC_{50} values for each period of exposure were calculated to explore the dose relationship to the death rate and quantify it. The toxicity data are illustrated in Table II.

Table II: Acute toxicity of d-trans allethrin and d-phenothrin to the zebrafish

Insecticide	Exposure Periods (h)	LC_{50} (µg/L)	Confidence Limit		Slope	Chi-square Values
			LCL	UCL		
	24	0.8	0.35	1.25	2.3083	0.067
d-trans allethrin	48	0.87	0.1	1.64	3.4625	0.06
	72	0.8	0.32	1.28	3.8953	0.074
	96	0.8	0.36	1.24	3.8953	0.074
	24	0.51	0.14	0.88	2.3168	0.067
d-phenothrin	48	0.56	0.07	1.05	3.4772	0.06
	72	0.51	0.2	0.86	3.9124	0.074
	96	0.51	0.23	0.79	3.9124	0.074

Linear Regression $r^2 = 0.067$

p-value > 0.05 between LC_{50} and exposure period

The results indicated that the LC_{50} values remained constant with increased exposure period, with $r^2 = 0.067$ ($p > 0.05$), indicating no significant correlation between the LC_{50} values and exposure periods. The chi-square values in the current study are not significant, indicating that the fish groups in the experiment were homogenous.

Zebrafish toxicity levels to human dose extrapolation

The Table III shown that the d-trans allethrin and d-phenothrin HED were 0.003 and 0.002 µg /kg and the MTD dosage were 0.18 and 0.12 µg /kg with the dosage required from stock insecticide solution were 16 and 17 µL respectively.

Table III: Human equivalent dose for average adult and the dosage required from stock insecticide solution

Insecticide	HED (µg /kg)	MTD for Average Adult (µg/day)	Dosage of Insecticide Required (µL)
d-trans allethrin	0.003	0.18	16
d-phenothrin	0.002	0.12	17

DISCUSSION

Zebrafish insecticide toxicity levels

It was also implied that the pyrethroid toxicity exhibited by the zebrafish increased with the increased of the exposed dosage. The observed characteristic can be determined as the behavioural and stress indicator of toxic environment exposure. The toxicosis characteristic presented by the intoxicated fish was similar to a previous study in which the fish demonstrated respiratory distress, loss of balance, and skin irritation (17).

It can be assumed that prolonged period of action potential triggered by the chemicals cause increased respiratory, locomotion, and mucous secretion by the fish. Thus, it supported the notion that insecticide exposure aggravates the current study's toxicosis symptoms.

The comparison of mortality rate established that the toxicities increased with prolonged exposure time as 96 h period of exposure determined to be the most toxic among the four-time intervals in the study. Thus, the sequence order for the mortality rate following the exposure period was: 96 h > 72 h > 48 h, and 24 h. The varied mortality rate between the exposure period suggested the influence of accumulation, bio-transformation, and excretion of toxic metabolites in the zebrafish metabolite pathways (17). The metabolite pathways can be compromised with prolonged exposure to pyrethroid, as it disrupts the acid and alkaline phosphate secretions in the gills and liver by altering cellular activities that result in gills and liver damage even death (18). Thus, it can be implied from the initial observation in the study that prolonged exposure of insecticide causes fish organ failure and death.

The slope values were steep for both chemicals, with d-phenothrin exhibiting greater values than d-trans allethrin, indicating that d-phenothrin required a lower dose to produce similar toxic effects. The result corresponds to the previous data suggesting that permethrin is the most potent insecticide, with lower LC_{50}, followed by d-phenothrin and d-trans allethrin (MVCAC, 19). The study also mentioned that the LC_{50} values of d-phenothrin and d-trans allethrin were 15.8 µg/L and 80 µg/L in the study on freshwater fish, which confirmed that d-phenothrin is more lethal than d-trans allethrin. The data confirmed the higher toxicity sensitivity of d-phenothrin than d-trans allethrin towards the zebrafish noted in the current study.

The present study showed that zebrafish are sensitive towards the insecticide mixture (d-trans allethrin and d-phenothrin). It also supports the notion of the broad-spectrum toxic nature of insecticide since it kills the non-target organism, eventually harming the users and the environment.

Zebrafish toxicity levels to human dose extrapolation and its relevance

The toxic dosage amount as described in Table III is within the range amount found in pyrethroids samples deposited from insecticide aerosol taken from the surface areas of furniture in a home environment in a previous study (20).

The study also claimed that between 0.008 to 0.507 μg/cm^2 of pyrethroid was detected on the table and floor areas after insecticide application (20). Another study mentioned that the deposited insecticide varied according to the types of surface, i.e., titles, leather, wood, and vinyl, with the amount found ranged between 7.1 to 657 μg/cm^2 (21). However, there is no study specifically referring to the actual deposited insecticide amount in a millilitre scale. Hence, a legitimate risk exposure exists when these substances were extensively used daily in the home environment, as shown by the insecticide required and the MTD of the average adult in the current study.

CONCLUSION

In conclusion, toxicity effects were observed in zebrafish model after exposure to insecticide mixture (d-trans allethrin and d-phenothrin). It is worth noticing that the toxic MTD extrapolated from the study was in the microgram (μg) level (0.18 and 0.12 μg/day for d-trans allethrin and d-phenothrin), which is slightly lower than the levels detected in a household environment. Hence, there is legitimate insecticide hazard exposure risk towards humans based on the toxicity level of this findings.

Declaration of Interest

The authors declare no conflict of interest.

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