JKKI: Jurnal Kedokteran dan Kesehatan Indonesia
Indonesian Journal of Medicine and Health
Journal homepage: https://journal.uii.ac.id/JKKI
P-ISSN 2085-4145 | E-ISSN 2527-2950

Differences in *livor mortis* in Wistar rats due to organophosphate induction and normal mortality: A randomized experimental study

Novianto Adi Nugroho[1]*, Muhammad Shokhiful Wafa Arya Wida Sena[2], Adji Suwandono[1]

[1]Department of Forensic, Faculty of Medicine, Sebelas Maret University, Surakarta
[2]Study Program of Physics, Sebelas Maret University, Surakarta

Original Article

ARTICLE INFO

Keywords:
organophosphates,
livor mortis,
Wistar rats,
toxicity

***Corresponding author:**
dranto@staff.uns.ac.id

DOI: 10.20885/JKKI.Vol15.Iss2art5

History:
Received: December 18, 2023
Accepted: August 27, 2024
Online: August 27, 2024

ABSTRACT

Background: Pesticide intoxication, a significant global health issue, particularly in developing nations, is often caused by the most toxic pesticides, organophosphates. These substances activate the sympathetic and parasympathetic nervous systems, producing a characteristic livor mortis. It is a secondary sign of death that can be used to estimate the time and cause of death.

Objective: This study aims to determine the difference in livor mortis due to organophosphate poisoning and ordinary death (decerebration) using Wistar rats.

Methods: From March to April 2023, we conducted an experimental study with a posttest-only control group at the Laboratory of Animal Experiments, Faculty of Medicine, Sebelas Maret University. We used 32 male Wistar rats weighing 150-200 g, divided into control and test groups. The test group received organophosphate diazinon 1.16 ml through a nasogastric tube, while the control group was decerebrated. The data was processed with univariate analysis and an independent t-test.

Results: There was a significant difference in the appearance and the persistence time of livor mortis between the control and test groups ($p < 0.05$). The color of livor mortis in the control group was purplish blue, while in the test group, it was reddish to blackish blue. Most of our samples displayed a distribution of livor mortis in the abdominal and dorsal regions, with a some displaying an abdominal distribution only.

Conclusion: Our study reveals significant differences in the appearance and persistence time, as well as the color and distribution of livor mortis between decerebrated and organophosphate-induced dead rats.

INTRODUCTION

Agriculture is paramount to nations, often regarded as a "national security" due to the indispensability of its products for human survival.[1] The global trade value of agricultural goods surged to $1.3 trillion in 2017, exhibiting a sevenfold increase in real terms compared to three decades ago.[2] To guarantee the efficiency of agricultural practices, pesticides are commonly applied. Despite existing regulations that manage pesticide usage, a lack of awareness and insufficient implementation mechanisms lead to the unregulated application of these substances, thereby contributing to human pesticide poisoning.[3]

Pesticide intoxication stands as a significant contributor to global mortality and morbidity, particularly in developing nations where agriculture serves as the primary commodity.[4] In 2020, globally, there were approximately 385 million annual cases of unintentional pesticide poisoning, resulting in around 11,000 fatalities. Furthermore, there are 110,000 to 168,000 deaths due to intentional pesticide poisoning, primarily in rural agricultural regions of low- and middle-income countries.[5] Pesticides can be categorized based on their chemical composition, including

organophosphates, carbamates, organochlorine, and chloralose compounds.[6] Organophosphates, being the most extensively utilized substances, are the primary contributors to fatal incidents globally.[7] As per reports, approximately 3 million individuals worldwide are exposed to organophosphates each year, leading to an annual mortality rate of 300,000.[8] Studies suggest that in developing nations characterized by underdeveloped healthcare systems and larger populations reliant on agriculture, a higher frequency of incidence is expected.[9]

Organophosphates can activate both the sympathetic and parasympathetic nervous systems. In fatality cases, respiratory failure is typically the predominant cause, resulting from bronchoconstriction, weakening (paralysis) of respiratory muscles, bronchorrhea, or central respiratory depression.[10] Inhibition of acetylcholinesterase leads to acute toxicity. Organophosphate intoxication can cause a range of clinical manifestations, such as muscarinic, nicotinic, and central nervous system effects.[11] These signs and symptoms can be remembered using the acronym DUMBELS: Diarrhea, Urination, Myosis, Bronchospasm, Emesis, Lacrimation, and Salivation.[12]

Diazinon is a frequently used organophosphate pesticide, impacting various pests by deactivating the acetylcholinesterase enzyme. Despite its widespread use, global concerns about the potential toxicity of diazinon to human health have arisen, particularly regarding its presence in food.[13] Furthermore, statistically, in rice fields, where more than 1,000,000 kg of granular pesticides and around 600,000 liters of liquid pesticides are applied, diazinon stands out as the predominant type of pesticide in use.[14]

Changes that occur in the body of a corpse are signs of death, categorized into primary (uncertain) and secondary (definite) signs.[15] Primary signs, which occur shortly after death, include the cessation of the cardiovascular, respiratory, and central nervous systems. On the other hand, secondary signs happen sometime after death, including a decrease in temperature (algor mortis), postmortem lividity (livor mortis), corpse stiffness (rigor mortis), decomposition, adipose, and mummification.[16] Secondary signs of death are usually used to estimate the time and cause of death.[17]

Livor mortis is the blood collection into the skin and subcutaneous tissue accompanied by dilation of capillaries.[15] It is a definitive sign of death and can help estimate the length of time of death, determine the position of the corpse at the time of death, and estimate the cause of death.[16] Several factors influence it, such as the duration of blood in liquid, blood volume capacity, and the color of livor mortis. The more blood volume, the faster the livor mortis and vice versa.[18] The color of livor mortis also indicates the cause of death. Brick red indicates carbon monoxide gas poisoning, bright red indicates cyanide poisoning, bluish brown indicates aniline and nitrobenzene poisoning, dark brown indicates phosphorus poisoning, dark red indicates asphyxia, and dark red bluish indicates organophosphate poisoning.[15,19,20]

Livor mortis, a vital element in forensic analysis, estimates the time and cause of death.[16] In our study, we focused on the duration of formation and persistence, color difference, and distribution of livor mortis, all of which were influenced by exposure to organophosphates.

The human corpse is an ideal sample, but because it is impossible, we used the Wistar rats without reducing the validity and scientificity. The effects of organophosphate toxicity have been widely studied, but the effects of organophosphate toxicity on livor mortis remain elusive. This study aims to determine the differences in livor mortis using the parameters of the duration of formation and persistence, color differences, and distribution of livor mortis found in the carcasses of organophosphate-intoxicated Wistar rats.

METHOD

Study design

From March to April 2023, we conducted an experimental study with a posttest-only control group at the Laboratory of Animal Experiments, Faculty of Medicine, Sebelas Maret University, to assess livor mortis in dead rats.

Population and sample

We used the male Wistar rats (*Rattus norvegicus*) aged 2-3 months, weighing 150-200 g. Each animal was monitored daily during the acclimatization period to ensure the achievement of inclusion criteria. The characteristics of the subjects were chosen for practical reasons, as well as their similarities with the human physiological

system.[21,22] We excluded the animals when in the adaptation phase for five days in the laboratory room, rats experienced signs of illness such as oily, falls out, and dull hair with piloerection; dramatic decreased of body weight; pallor, dehydration, abnormal posture; eyelids slightly closed, sunken eyes, exophthalmos, red secretions around the eyes; diarrhea or blood in the stool; tremor, coma, ataxia; sneezing, tachypnea, breath sounds, nasal discharge. Regular checking was carried out daily at regular intervals.[23]

Data collection

The sample size was determined using Federer's formula. In this study, we categorized into two groups: control or decerebration (n = 16) and test or organophosphate induction groups (n = 16).[24] Livor mortis was observed every 5 minutes after death until there was a color change, which was then recorded as the duration of livor mortis appearance. Then, it is followed by pressing the lividity to see it persist. We also observed the color and distribution of livor mortis. Each test animal was divided into three testing sessions and divided into six rats per session for each group. Data on the duration of livor mortis appearance, persistence, color, and distribution were observed periodically for five minutes for 1-2 days postmortem. In addition, data on treatment time and time to death were also observed. The observation site used a container with a length and width of 70 cm x 40 cm filled with 5-6 rats.

Research variables and measurement

We used the organophosphate treatment as an independent variable, in which we used diazinon (Diazinon® liquid 600 EC 100 ml). The liquid was administered to animals through a nasogastric tube (1.16 ml/administration). This administration dose was determined based on Nair and Jacob's dose conversion guide.25 The maximum dose in rats for diazinon ranges from 250 to 1,250 mg/kg body weight (BW). Since we used 200 g of rats, we obtained the optimal dose of 350 mg/kg BW. Then, the concentration of the solution was obtained from the net weight of diazinon, which was 10% multiplied by 600 mg/ml (Diazinon preparations on the market) or 60 mg/ml. Therefore, the induction volume (ml) is (0.2 kg x 350 mg/kg) / (60 mg/ml) = 1.16 ml.

The livor mortis was assessed using four parameters. The appearance and persistence of livor mortis were measured using a ratio scale, while the color and distribution of livor mortis were measured using a nominal scale.

Data analysis

This study used an independent t-test for quantitative observations and comparisons.[26] The data were examined using IBM Statistical Package for the Social Sciences (SPSS) Statistics Version 25. We used the Shapiro-Wilk test for parametric data with a sample size of less than 50. The homogeneity test will be analyzed using Levene's Test.

Ethics

The Health Research Ethics Commission of Dr. Moewardi Hospital granted ethical approval for this research under reference number 367/III/HREC/2023.

RESULT

We discovered a significant difference in the duration of livor mortis appearance between the control and test groups (p = 0.001) (Table 1). The mean duration of the livor mortis appearance in organophosphate-induced dead rats was faster (26.25 minutes) than ordinary dead rats (63.81 minutes). We also found that the mean time of livor mortis persistence in the test group was faster than the control group (260.94 and 343.75 minutes, respectively), with p = 0.001. In macroscopic observation, the color of livor mortis in the control group was all purplish blue, and the test group was dominated by reddish blue (Figures 1 and 2). In the distribution of livor mortis, most of the control and test groups were distributed in the abdomen and back (Table 2, Figure 3, and Figure 4).

DISCUSSION

Medical science defines humans as individuals and cells, while death can be defined as individual (somatic) and intracellular death.[27] The central nervous, cardiovascular, and respiratory systems stop functioning permanently (irreversibly) during somatic death, marked by no presence of body reflexes, heartbeat, movement, or breath sounds. However, in somatic death, metabolism continues with the remaining oxygen supply.[28] The endurance of each tissue and organ is not similar in response to the absence of oxygen, so each tissue

Table 1. Result of livor mortis observations

	Livor mortises appear (minutes)		Persistent livor mortis (minutes)	
	Decerebration	**Organophospat**	**Decerebration**	**Organophospat**
Mean±SD*	63.81±6.38	26.25 ±7.54	343.75±67.32	260.94±44.32
p	0.001		0.001	

*Normal data distribution from Shapiro-Wilk test, p > 0.05, SD: standard deviation, p from independent t-test

Figure 1. Livor mortis in the control group (left) and organophosphate group (right)

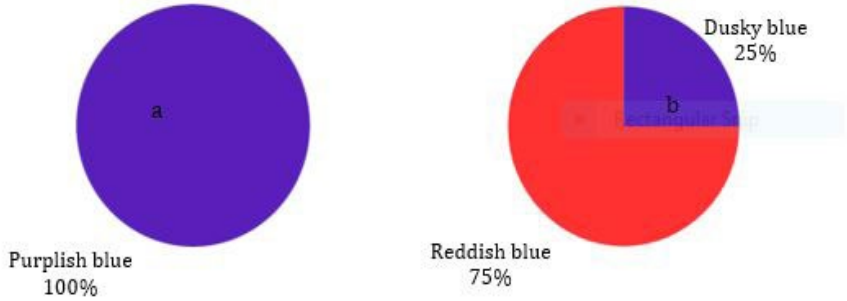

Figure 2. Comparison of the color of livor mortis in the control (a) and test groups (b)

Figure 3. Livor mortis in the control (left) and organophosphate groups (right)

Table 2. Result of distribution of livor mortis observations

Sample	Decerebration	Organophosphate
1	abdomen+back	abdomen+back
2	abdomen+back	abdomen+back
3	abdomen	abdomen+back
4	abdomen+back	abdomen
5	abdomen	abdomen
6	abdomen+back	abdomen+back
7	abdomen	abdomen+back
8	abdomen+back	abdomen+back
9	abdomen	abdomen
10	abdomen+back	abdomen+back
11	abdomen	abdomen
12	abdomen	abdomen+back
13	abdomen+back	abdomen
14	abdomen+back	abdomen+back
15	abdomen	abdomen
16	abdomen+back	abdomen+back

organ has a different duration of cellular death.[27]

It is necessary to examine the signs and changes that occur after death. Signs of death can be divided into uncertain (primary) and certain (secondary) death.[28] Primary signs of death are associated with somatic (systemic death), which is associated with irreversible cessation of vital functions of the brain, heart, and lungs.[15] Secondary signs of death are known as definite signs and occur at the molecular level. Depending on the capacity of cells and tissues to survive without oxygen, somatic and molecular death do not co-occur. After death, a sign of secondary death can be found in the corpse, such as a decrease in body temperature (algor mortis), bruising (livor mortis), stiffness (rigor mortis), and decomposition.[28]

In this study, we concentrated on macroscopic alterations, particularly variations in the sign of secondary death in rat carcasses induced by organophosphate and those that died naturally. Our experiments aimed to validate the fundamental understanding of observable and time-dependent post-mortem interval (PMI) findings in rats affected by organophosphate and those that died naturally. Remarkably, both control and experimental rat carcasses observed a spectrum of alterations.

Organophosphates are among the most toxic pesticides and often result in human poisoning.[29] Organophosphates are skin contact, stomach, and inhalation toxicants.[30] They can kill in small amounts, but adults need more than a few milligrams for death to occur. Direct inhibitory organophosphates are organophosphates that do not require metabolic activity to cause toxic effects in direct contact areas, such as sweating (skin contact), bronchospasm (respiratory contact), and miosis or pinpoint pupils (eye contact).[31] The need for metabolic activation prior to acetylcholinesterase inhibition caused by organophosphates is divided into direct inhibitory (containing = O) and indirect inhibitory (containing = S) organophosphates. Indirect inhibitory organophosphate compounds must undergo bioactivation to become biologically active. Parathion, diazinon, malathion, and chlorpyrifos are indirectly inhibited organophosphate compounds that are more toxic than their parent compounds.[32]

The fatal doses for the organophosphate group are parathion 10 mg/kg BW, malathion 1-5 g, tetraethyl pyrophosphate 0.4 mg/kg BW, and systox 100 mg. Several factors influence the action of toxins, so the lethal dose is difficult to determine.[33] Therefore, the toxic dose used is the Approximately Fatal Dose (AFD), which helps doctors assess a case's prognosis. Approximate fatal dose may also be referred to as Usual Fatal Dose (UFD).

Usually, the UFD is based on the Minimum Lethal Dose (MLD), which is generally indicative of the lethal dose in 50% of animals (LD50). The UFD for diazinon-type organophosphates is 0.1 g/kg BW in adults and 250-1,250 mg/kg BW in rats.[34] Calculations were done, and the lethal dose in Wistar rats was 1.16 ml.

The test animals are defined as dying when the central nervous, cardiovascular, and respiratory systems permanently stop, which can be referred to as somatic death. Individual death can be a marker of subsequent intracellular death.[35] The initial postmortem alterations happen during the early stages when the body remains fresh, before the deterioration of soft tissues, encompassing rigor mortis and livor mortis. These changes hold forensic importance. Livor mortis (lividity or postmortem hypostasis) refers to blood pooling towards the body's lower side due to gravitational forces acting upon blood and bodily fluids.[36]

The control group exhibited a significantly longer average forming time of livor mortis (64 minutes) and a significantly longer mean disappearing time (344 minutes) compared to the treatment group (26 minutes and 261 minutes, respectively). These results indicated that livor mortis manifested and persisted more rapidly in rats induced with organophosphates than in those that died naturally. A study in Manado, using five rabbits for each control and experimental group, noted a non-significant discrepancy in the onset and duration of livor mortis.[37]

In humans, a livor mortis spot is formed within 20-30 minutes after death.[20] In 1-2 hours postmortem, it begins to be visible. After 2-4 hours, more prominent spots begin to be detectable. Consequently, homogeneous spots are formed in 4-6 hours postmortem, and livor mortis begins to remain in 8-12 hours. After 8-12 hours, it will not disappear by applying pressure.[38] The disappearance of livor mortis by suppression indicates the lividity has not been completely fixed.[18]

The appearance and persistence of livor mortis in rats are faster than in humans due to the smaller body area of rats, which allows for faster formation when circulation failure occurs. In humans, the speed of livor mortis progression increases during rapid decomposition but is slowed down in cold environmental conditions, remaining consistent within 24 to 36 hours. However, the manifestation of livor mortis varies significantly depending on several factors, including environmental conditions, location, species, body size, patterns of color change, and circumstances surrounding death.[36]

Acute organophosphate poisoning, particularly diazinon, poses a severe threat as it can induce cholinergic syndrome. This syndrome results from the enzyme acetylcholinesterase being inhibited by phosphorylation, leading to an increase in acetylcholine levels. The broad effects of this syndrome, such as bronchospasm, dyspnea, vasoconstriction, tachycardia, hypertension, muscle fasciculation, and muscle cramps, can lead to respiratory failure.[11] The failure is characterized by paralysis of respiratory muscles, respiratory center depression, pulmonary edema, excessive bronchial secretions, and bronchoconstriction, leading to asphyxia that causes treated rats to die. If the individual survives the immediate poisoning incident, they will have a risk of long-term complications.[39] Cholinergic syndrome is commonly associated with organophosphates, parasympathomimetic drugs, carbamate poisoning, and certain types of fungi.[40]

In asphyxia, the lungs lack oxygen, leading to increased carbon dioxide levels and fibrinolysin activity. This increase in fibrinolysin activity makes blood flow easy and makes it challenging to coagulate.[41] The source of this fibrinolysin is unknown. However, it most likely originates from the vascular endothelium and the serous surface of the pleura. The activity of fibrinolysin is very evident in capillaries filled with blood so that the livor mortis will settle quickly. In asphyxia death, blood will contain high fibrinolysin due to the process of oxygen insufficiency so that livor mortis will form and settle faster.[42]

Macroscopic observation found that the corpse was purplish blue in the control group, while three-quarters of the samples were reddish blue, and a quarter were blackish blue in the test group. Our results are aligned with the theory that in asphyxia conditions, the amount of carbon dioxide in the blood increases, causing cyanosis, and the color of the livor mortis formed is dark red-bluish.[43] In a separate investigation using a rabbit specimen, livor mortis appeared purplish-blue in the control group and reddish-purple in the test group. Discrepancies between these two studies could derive from variations in sample size and

composition across the studies.[37]

The visible red color can be caused by bleeding spots (petechiae) or, in the case of asphyxia, it is commonly referred to as Tardieu's spot. It occurs due to damage to the capillary endothelial wall and increased capillary permeability because of hypoxia. Due to local dilatation of blood capillaries, these bleeding spots often form in loose connective tissues, such as tissues under the eyelids and organs with transparent membranes.[44] The abdomen of rats has thinner skin and many blood vessels, which makes the color redder due to dilation of local capillary. In this study, the color of the livor mortis in the control group was purplish blue, which is discordance with the theory that the normal color of livor mortis is reddish purple. This color is formed due to the separation of hemoglobin with oxygen and continuous oxygen consumption. The longer the postmortem interval, the darker the color will look due to the formation of deoxyhemoglobin.[15] Livor mortis color indicates the cause of death, such as brick red color indicates carbon monoxide gas poisoning, bright red color indicates cyanide poisoning, bluish brown indicates aniline and nitrobenzene poisoning, dark brown indicates phosphorus poisoning, and dark red indicates asphyxia.[45] In addition, the foam will appear in the mouth of rats because of organophosphate induction, which is related to the cholinergic syndrome condition. Organophosphates, which hinder the function of the enzyme acetylcholinesterase, lead to the breakdown of the neurotransmitter acetylcholine. Consequently, there is a surplus of acetylcholine in the body, resulting in increased nerve stimulation throughout different body regions, including surrounding the mouth and respiratory passages. This excessive stimulation can trigger involuntary muscle contractions in the mouth and respiratory passages, forming foam.[28]

This study also found that the distribution of livor mortis in both groups is more than 50% in the abdominal and back. However, in the test group, the percentage was more significant. The distribution of livor mortis depends on the corpse's position.[46] According to the law of gravity, blood will collect in the lowest part of a person's body when death occurs. Capillary blood vessels will be restrained by the continuous flow of blood, which will cause blood cells to escape from the capillary blood vessels into the surrounding tissues, giving the impression of color. However, this does not happen for body parts pressed against hard mats.[47] The increased activity of fibrinolysin and carbon dioxide, which makes it difficult for blood to coagulate and flow, contributes to the widespread distribution of postmortem lividity. These results are also consistent with the broader distribution of postmortem lividity in the test group, in which we found both distributions in the abdomen with back about 62.5%.

There are still some limitations in this study. The dose variation in this study only uses one administration time. Therefore, we cannot determine the optimal lethal dose in the death of test animals. Also, this study was conducted periodically due to limited human resources, and there may be bias related to testing time. The use of ketamine anesthesia at a dose of 0.3 per oral in the samples is a confounding factor in meeting the principles of animal welfare. Although the use of this substance did not influence the results, further study is still needed.

CONCLUSION

We discovered a significant difference in the appearance and persistence duration of livor mortis between ordinary (decerebrated) and organophosphate-induced dead rats. We also found distinct color variations of livor mortis between ordinary dead rats (purplish blue) compared to organophosphate poisoning (dark red bluish). Most of our samples displayed a distribution of livor mortis in the abdominal and dorsal regions, with a some displaying an abdominal distribution only. Further studies with more subjects and proper anesthesia methods are needed for better results that represent our hypothesis.

CONFLICT OF INTEREST

There is no conflict of interest.

ACKNOWLEDGEMENTS

The author would like to thank the Department of Forensic and LPPM (*Lembaga Penelitian dan Pengabdian kepada Masyarakat*) of Sebelas Maret University for making this study possible.

AUTHOR CONTRIBUTIONS

NNA and MSWAWS designed the study concept. NNA formulated the theory and conducted the

calculations. MSWAWS and SA validated the analytical methods. NNA motivated MSWAWS to explore and oversee the discovery process. All authors participated in results and discussions and contributed to the final manuscript.

LIST OF ABBREVIATIONS

AFD: Approximately Fatal Dose; UFD: Usual Fatal Dose; MLD: Minimum Lethal Dose; LD50: Lethal Dose 50.

REFERENCES

1. Beckman J, Countryman AM. The importance of agriculture in the economy: Impacts from COVID-19. Am J Agric Econ. 2021;103(5):1595–1611. DOI: https://doi.org/10.1111/ajae.12212
2. Zhao X, Wise MA, Waldhoff ST, Kyle GP, Huster JE, Ramig CW, et al. The impact of agricultural trade approaches on global economic modeling. Glob Environ Change. 2022;73:1–15. DOI: https://doi.org/10.1016/j.gloenvcha.2021.102413
3. Tudi M, Daniel RH, Wang L, Lyu J, Sadler R, Connell D, et al. Agriculture development, pesticide application and its impact on the environment. Int J Environ Res Public Health. 2021;18(3):1112. DOI: https://doi.org/10.3390/ijerph18031112
4. Tyler J, Kris B, Roshana S, Anmol S, Shaza A. Organophosphate poisoning and suicide in Nepal: A reflection on the limitations of behavioral health resources. Int J Crit Care Emerg Med. 2020;6(1):1–3. DOI:https://doi.org/10.23937/2474-3674/1510097
5. Moebus S, Boedeker W. Case fatality as an indicator for the human toxicity of pesticides-a systematic scoping review on the availability and variability of severity indicators of pesticide poisoning. Int J Environ Res Public Health. 2021;18(16):8307. DOI: https://doi.org/10.3390/ijerph18168307
6. Daraban GM, Hlihor RM, Suteu D. Pesticides vs. biopesticides: From pest management to toxicity and impacts on the environment and human health. Toxics. 2023;11(12):983. DOI: https://doi.org/10.3390/toxics11120983
7. Mlayeh S, Annabi K, Daly AB, Jedidi M, Dhiab MB. Pesticide poisoning deaths: A 19-year retrospective study of medicolegal autopsies in center tunisia. Egypt J Forensic Sci. 2020;26. DOI: https://doi.org/10.1186/s41935-020-00201-7
8. Kaushal J, Khatri M, Arya SK. A treatise on organophosphate pesticide pollution: Current strategies and advancements in their environmental degradation and elimination. Ecotoxicol Environ Saf. 2021;207:111483. DOI: https://doi.org/10.1016/j.ecoenv.2020.111483
9. Omar S, Bahemia IA, Toerien L, San Pedro KM, Khan AB. A retrospective comparison of the burden of organophosphate poisoning to an intensive care unit in Soweto over two separate periods. Afr J Emerg Med. 2021;11(1):118–122. DOI: https://doi.org/10.1016/j.afjem.2020.09.007
10. Hiremath P, Rangappa P, Jacob I, Rao K. Pseudocholinesterase as a predictor of mortality and morbidity in organophosphorus poisoning. Indian J Crit Care Med. 2016;20(10):601–604. DOI: https://doi.org/10.4103/0972-5229.192052
11. Aman S, Paul S, Chowdhury FR. Management of organophosphorus poisoning: Standard treatment and beyond. Crit Care Clin. 2021;37(3):673–686. DOI: https://doi.org/10.1016/j.ccc.2021.03.011
12. Lorke DE, Petroianu GA. The experimental oxime K027-A promising protector from organophosphate pesticide poisoning. A review comparing K027, K048, pralidoxime, and obidoxime. Front Neurosci. 2019;13:427. DOI: https://doi.org/10.3389/fnins.2019.00427
13. Nematollahi A, Rezaei F, Afsharian Z, Meybodi NM. Diazinon reduction in food products: A comprehensive review of conventional and emerging processing methods. Environ Sci Pollut Res Int. 2022;40342–40357. DOI: https://doi.org/10.1007/s11356-022-19294-9
14. Ghanbari F, Moattar F, Monavari SM, Arjmandi R. Human health risk assessment of organophosphorus pesticide in rice crop from selected districts of Anzali International Wetland basin, Iran. Hum Exp Toxicol. 2017;36(5):438–444. DOI: https://doi.org/10.1177/0960327116657603
15. Shedge R, Krishan K, Warrier V, Kanchan T. Postmortem changes. In StatPearls [internet]. StatPearls publishing; 2022 [cited 2023 Dec 12]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539741/
16. Almulhim AM, Menezes RG. Evaluation of

postmortem changes. In: StatPearls [internat]. StatPearls publishing; 2023 [cited 2024 Jan 11]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554464/
17. Shrestha R, Kanchan T, Krishan K. Methods of estimation of time since death. In StatPearls [internet]. StatPearls publishing; 2023 [cited 2023 Dec 12]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK549867/
18. Byard RW, Gilbert JD. Patterned lividity - the 'underwear sign'. Forensic Sci Med Pathol. 2022;18(3):269–270. DOI: https://doi.org/10.1007/s12024-022-00485-2
19. Hayman J, Oxenham M. Human body decomposition. Academic Press. 2016; Pages 1-12. DOI: https://doi.org/10.1016/B978-0-12-803691-4.00001-7
20. Burkhard M. Encyclopedia of forensic sciences, Third Edition. Elsevier. 2023; Pages 193-205. DOI: https://doi.org/10.1016/B978-0-12-823677-2.00011-8
21. Bailey J, Thew M, Balls M. An analysis of the use of animal models in predicting human toxicology and drug safety. Altern Lab Anim. 2014;42(3):181–199. DOI: https://doi.org/10.1177/026119291404200306
22. Bhadaniya AR, Kalariya VA, Joshi DV, Patel BJ, Chaudhary S, Patel HB, et al. Toxicopathological evaluation in wistar rats (*Rattus norvegicus*) following repeated oral exposure to acephate. Toxicol Ind Health. 2015;31(1):9–17. DOI: https://doi.org/10.1177/0748233712468017
23. Kupferschmid BJ, Therrien B, Rowsey PJ. Different sickness responses in adult and aged rats following lipopolysaccharide administration. Biol Res Nurs. 2018;20(3):335–342. DOI: https://doi.org/10.1177/1099800418759599
24. Rukmana A, Supardi LA, Sjatha F, Nurfadilah M. Responses of humoral and cellular immune mediators in BALB/c mice to LipX (PE11) as seed tuberculosis vaccine candidates. Genes (Basel). 2022;13(11):1954. DOI: https://doi.org/10.3390/genes13111954
25. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7(2):27–31. DOI: 10.4103/0976-0105.177703
26. Kim HY. Statistical notes for clinical researchers: The independent samples t-test. Restor Dent Endod. 2019;44(3):e26. https://doi.org/10.5395/rde.2019.44.e26
27. Green DR, Llambi F. Cell death signaling. Cold Spring Harb Perspect Biol. 2015; 7(12). https://doi.org/10.1101/cshperspect.a006080
28. Adeyinka A, Kondamudi NP. Cholinergic crisis. In StatPearls [Internet]. StatPearls Publishing; 2023 [cited 2024 May 10]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482433/
29. Naughton SX, Terry AV. Neurotoxicity in acute and repeated organophosphate exposure. Toxicology. 2018; 408, 101–112. https://doi.org/10.1016/j.tox.2018.08.011
30. Kumar S, Kaushik G, Villarreal-Chiu JF. Scenario of organophosphate pollution and toxicity in India: A review. Environ Sci Pollut Res Int. 2016; 23(10), 9480–9491. https://doi.org/10.1007/s11356-016-6294-0
31. Yan Z, Jin X, Liu D, Hong Y, Liao W, Feng C & Bai Y. The potential connections of adverse outcome pathways with the hazard identifications of typical organophosphate esters based on toxicity mechanisms. Chemosphere. 2021; 266, 128989. https://doi.org/10.1016/j.chemosphere.2020.128989
32. Bhatt P, Zhou X, Huang Y, Zhang W, & Chen S. Characterization of the role of esterases in the biodegradation of organophosphate, carbamate, and pyrethroid pesticides. J Hazard Mater. 2021; 411, 125026. https://doi.org/10.1016/j.jhazmat.2020.125026
33. Biswas G. Review of forensic medicine and toxicology: Including clinical and pathological aspects: MCQs of previous years PG entrance examinations included (2nd ed.). Jaypee. 2012. DOI: https://doi.org/10.5005/jp/books/12589
34. Elmazoudy RH, Attia AA, Abdelgawad HS. Evaluation of developmental toxicity induced by anticholinesterase insecticide, diazinon in female rats. Birth Defects Res B Dev Reprod Toxicol. 2011;92(6):534–542. DOI: https://doi.org/10.1002/bdrb.20322
35. Miller MA, Zachary JF. Mechanisms and morphology of cellular injury, adaptation, and death. Pathologic Basis of Veterinary Disease, 2–43.e19. DOI: https://doi.org/10.1016/B978-0-323-35775-3.00001-1
36. Boonmayaphan M, Butrat P. Postmortem macroscopic changes in rats under rat house conditions. Journal of Applied Animal Science. 2022; 15(1), 9–22. https://he02.tci-thaijo.org/index.php/jaas_muvs/article/

view/257108
37. Thanos CAA, Tomuka D, Mallo NTS. Livor mortis pada keracunan insektisida golongan organofosfat di kelinci. E-CliniC. 2016;4(1). DOI: https://doi.org/10.35790/ecl.v4i1.10827
38. Byard RW. Estimation of the time since death. Academic Press. 2020; Pages 11-27. DOI: https://doi.org/10.1016/B978-0-12-815731-2.00002-9
39. Giyanwani PR, Zubair U, Salam O, Zubair Z. Respiratory failure following organophosphate poisoning: A literature review. Cureus. 2017;9(9):e1651. DOI: https://doi.org/10.7759/cureus.1651
40. Vale A, Lotti M. Organophosphorus and carbamate insecticide poisoning. Handbook of clinical neurology. 2015; 131, 149–168. https://doi.org/10.1016/B978-0-444-62627-1.00010-X
41. Schwameis M, Schober A, Schörgenhofer C, Sperr WR, Schöchl H, Janata-Schwatczek K, et al. Asphyxia by drowning induces massive bleeding due to hyperfibrinolytic disseminated intravascular coagulation. Crit Care Med. 2015;43(11): 2394–2402. DOI: https://doi.org/10.1097/CCM.0000000000001273
42. Donaldson AE, Lamont IL. Biochemistry changes that occur after death: Potential markers for determining post-mortem interval. PLoS One. 2013;8(11):e82011. DOI: https://doi.org/10.1371/journal.pone.0082011
43. Pahal P, Goyal A. Central and peripheral cyanosis. In: StatPearls [Internet]. StatPearls Publishing; 2022 [cited 2024 Jan 11]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559167/
44. Bugelli V, Campobasso CP, Angelino A, Gualco B, Pinchi V, Focardi M. Postmortem otorrhagia in positional asphyxia. Am J Forensic Med Pathol. 2020;41(3):217–219. DOI: https://doi.org/10.1097/PAF.0000000000000559
45. Lalit PC, Mamta P. Assessment and diagnosis of poisoning with characteristics features in living or dead. J Forensic Sci & Criminal Inves. 2018; 10(4): 555796. 10.19080/JFSCI.2018.10.555796.
46. Leonard A, Vink R, Byard RW. Brain fluid content related to body position and postmortem interval - An animal model. J Forensic Sci. 2016;61(3):671–673. DOI: https://doi.org/10.1111/1556-4029.13038
47. Madea B. Methods for determining time of death. Forensic Sci Med Pathol. 2016;12(4):451–485. DOI: https://doi.org/10.1007/s12024-016-9776-y