El-Hayah Vol. 9, No.4 (2024) 181-193

El-Hayah

JURNAL BIOLOGI
Journal Homepage: http://ejournal.uin-malang.ac.id/index.php/bio/index
e-ISSN: 2460-7207, p-ISSN: 2086-0064

Original research article

Study of fly's behavior as a parameter of the impact of toxic compounds on living things

Siti Zubaidah[1], Deny Setiawan[1], Muhamad Justitia Ramadhan[1], Natasya Adiba Zahrah[1], Sinta Kharomah[1], Wachidah Hayuana[1], Muhammad Iqbal Najib Fahmi [1], Ahmad Fauzi[2*]

[1]*Universitas Negeri Malang, Jl. Semarang No. 5, Malang 65145, Indonesia*
[2]*Universitas Muhammadiyah Malang, Jl. Raya Tlogomas 246 Malang 65144, Indonesia*

**Corresponding author*
Email: ahmad_fauzi@umm.ac.id
DOI: 10.18860/elha.v9i4.29974

Article Info

Article history:
Received 21 November 2023
Received in revised form 04 February 2024
Accepted 27 March 2024

Key Word:
Drosophila, Fly behavior, Toxicology,

Abstract

Various toxic compounds still often contaminate the environment and food of living things to this day. Drosophila is often used as a model organism to study the negative effects of exposure to toxic compounds on organisms. The purpose of this systematic literature review (SLR) is to analyze the distribution, contribution, and gap analysis of studies reporting the effect of toxic compounds on behavior in Drosophila. After conducting a search in the Scopus database, 57 titles that matched the entered search query were obtained. After selection and evaluation step, a total of 19 Scopus indexed articles that met the inclusion and exclusion criteria were successfully collected for analysis. The three countries that most frequently research Drosophila behavior are the US, China, and Nigeria. A total of 5 clusters resulted from the results of bibliometric analysis. Various behavioral studies have included developmental variables, gene expression, to the Circadian clock. Toxic compounds that are often studied generally come from the group of metal compounds. On the other hand, multigenerational studies to analyze the long-term effects of toxic compounds and the plasticity of phenotypic changes into gap analysis have been successfully identified.

1. INTRODUCTION

Research on toxicity has garnered substantial attention in the scientific community over time [1,2]. Toxicity studies aim to comprehend the adverse effects of various substances on living organisms, providing insights into the impact of these substances on health and the environment. Various researchers still focus their research on toxicology because of the high exposure to toxic substances that still frequently occurs in various regions [3,4]. Through studies that examine toxicity, researchers can also identify and confirm substances whose dangerous level is still unclear [5,6]. Apart from that, research in this field is also able to examine and evaluate various other compounds that are able to suppress the negative impact of toxic substances on the body.

Various toxicological studies often require model organisms to study the toxic properties of the substances being studied. Of the various organisms that exist, Drosophila melanogaster is one of the popular model organisms that is often involved in research on toxic substances in various countries [7,8,9]. The various characteristics of this insect are the reason for Drosophila's popularity as a model organism in the field of toxicology, such as a fast life cycle, not requiring a large culture space, and culture costs that are not too expensive [10,14]. In addition, the genetic constitution of Drosophila is also similar to humans so that this organism is able to credibly model various human biological conditions [12]. Furthermore, due to the development of molecular biology techniques, researchers are also able to manipulate the genetic material or genetic expression of Drosophila [15,16,17] so that it will be easier for researchers to evaluate the impact of toxic substances in more depth.

Behavioral assays have become a staple in research when utilizing Drosophila as a research subject [18,19,20]. The intricate behaviors exhibited by this model organism provide unique insights into the subtle and often complex effects of toxic substances on an organism's physiology and nervous system [21,22,23]. These assays encompass various activities, ranging from locomotion [24,25] and feeding behavior [26,27] to circadian rhythms [28]. By observing behavioral responses in Drosophila, researchers can delve deeper into the behavioral responses induced by toxic substances, data that would be challenging to obtain in toxicity research solely relying on traditional toxicological assays.

Despite numerous toxicity studies involving behavioral assays in Drosophila, there has been no attempt to comprehensively map and review the entirety of findings related to fly behavior across various toxicity studies. On the other hand, a systematic literature review (SLR) focused on these studies can identify significant gaps in this field. This SLR will bridge these gaps by systematically analyzing and synthesizing available reputable literature. Furthermore, this review underscores the urgency of investigating toxicology with a behavior-centric perspective, given the increasing significance of behavioral endpoints in assessing the subtle yet critical impacts of toxic compounds on organisms and ecosystems. This, in turn, contributes to more accurate risk assessments and better-informed decision-making in the realms of environmental and human health.

2. MATERIALS AND METHODS

In this systematic literature review (SLR), we adhere to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, as depicted in Figure 1. The central research question driving this SLR is as follows: 'What diverse range of behaviors have researchers observed while investigating the toxicity using Drosophila?' We have chosen the Scopus database for this study, encompassing publications that incorporate Drosophila Behavior Assays (DCA) as one of their primary data sources. The specific search query employed is presented in Table 1, along with detailed inclusion and exclusion criteria outlined in the same table. Subsequently, our paper screening process commences, guided by the predetermined inclusion and exclusion criteria as outlined in Table 1.

Table 1. Search query, inclusion criteria, and exclusion criteria used

Component	Description
Search query	TITLE-ABS-KEY ("toxic substance" OR "toxic chemical" OR "pollutant" OR "toxicant" OR "toxic agent") AND ALL ("behavior" OR "behaviour") AND TITLE-ABS-KEY ("Drosophila" OR "fruit fly")
Inclusion criteria	articles that were published prior to 2023, classified as journal articles, in the final publication stage, sourced from journals, written in English, and available as open access.
Exclusion criteria	paper reviews, full papers that were inaccessible, non-original article

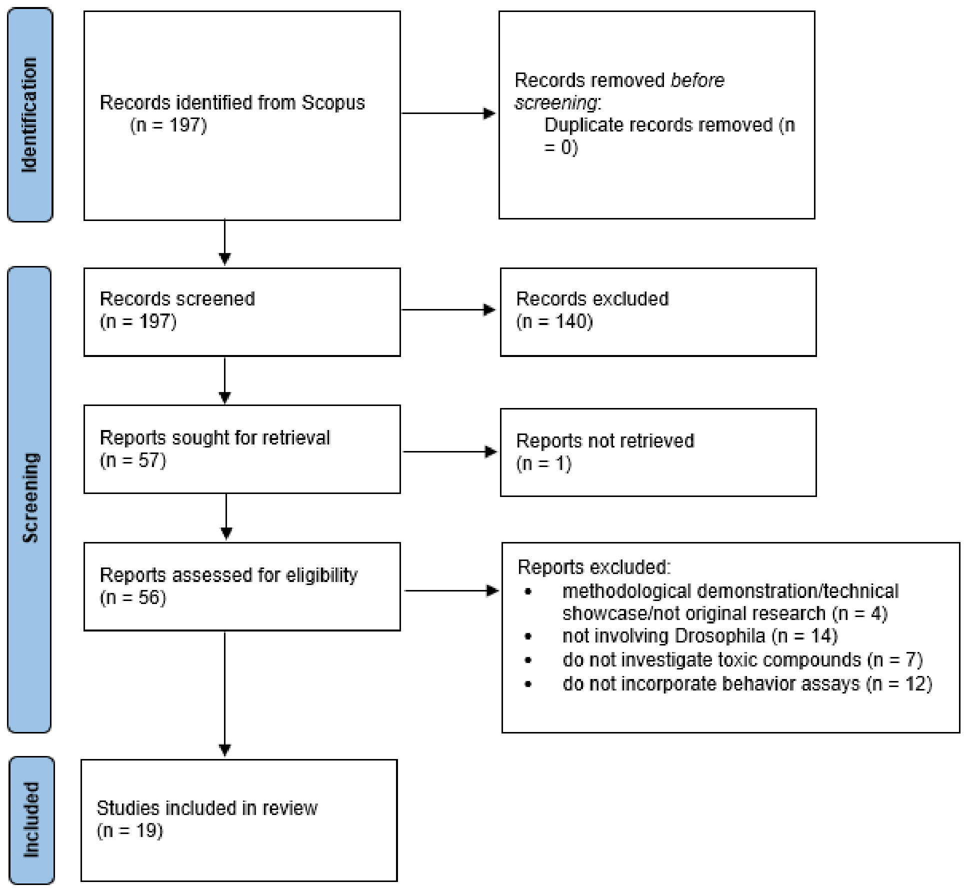

Figure 1. . PRISMA steps in this SLR study

3. Results

The PRISMA process has left us with 19 papers that are eligible for systematic review. Based on the provided methods, these papers can be categorized into two broad groups: those that aim to assess the toxicity of specific substances and those that aim to test other substances to mitigate the effects of exposure to toxic compounds. Two papers fall into the first group, while the rest belong to the second group.

The first theme to be examined as part of the data analysis in this SLR is the variety of toxic compounds under investigation. The data extraction results for this theme are presented in Table 2. A comprehensive analysis of the selected papers has revealed the remarkable diversity of toxic substances that have been tested using behavior assays in Drosophila. These findings indicate the versatile application of Drosophila behavior assays in the field of toxicology. Among the various toxic substances, the group most frequently tested includes heavy metals such as cadmium, mercury, copper, zinc, vanadium, iron, lead, and manganese. The frequent selection of heavy metals as the focus of studies is because this group of substances is considered environmental pollutants with significant impacts on ecosystems and public health. Furthermore, the higher prevalence of research related to mercury compared to other heavy metals may be attributed to the ongoing global issue of mercury contamination, which remains challenging to address effectively.

Table 2. Summary of extracted information for the theme of various toxic compounds

Substance Group	Tested Substance	No Paper
Heavy Metal	Cadmium	[29, 30]
	Mercury	[28],31,32,33, 34]
	Copper	[26,27]
	Zinc	[27]
	Vanadium	[35]
	Iron	[35]
	Lead	[36]
	Manganese	[24]
Organic Compound	4-Vinylcyclohexene 1,2-Monoepoxide	[37]
	4-Vinylcyclohexene Diepoxide	[37]
	Chlordane	[38]
	Caffeine	[39]
	Toluene	[40,41]
	Formaldehyde	[40]
	Dibutyl Phthalate	[42]
Polymer	Star Polycation	[25]
	Microplastics	[30]

Table 3. Summary of information extraction results for the variety of behavior assay theme

Category	Behavior Assay	Parameter
Feeding Behavior	Larva Mouth Hook Movement	number of mouth hook movements per minute [24]
	Larvae Two-Choice Gustatory Assay	Number of larvae choosing control food [39]
	Larva One-Choice Olfactory Assay	Larval movement in response to attractive odor [39]
	Larva Food Intake Assay	Reduction in larval food intake [39]
	Feeding Avoidance Assays	Concentration of marker consumed [26,39]
	Two-Color Choice Feeding Assay	Percentage of food preference [27]
	Proboscis Extension Reflex Assay	the fraction of full proboscis extension [27]
	Capillary Feeder Assay	Total consumption [27,42]

Locomotion	Larva Locomotion	Body Wall Contractions per minute [24]
	Imago Locomotion	Speed [40], Acceleration [40], Duration of Stops [40], Locomotion Rate [40], Meandering [40], Number of Slips [40], Duration of Rest [40], Number of Photobeam Breaks [41,42]
	Climbing behavior	Climbing Speed [32,35,38]
		Climbing Height [28,30]
		Number of flies climbing [25,29,30,35,37,38]
Flight Behavior	Flight Assay	Landing height [33,34]
Sleep Behavior	Sleep Duration	Sleep Duration [42]
	Sleep-Walking Assay	Number of times flies cross [28]
Reproductive Behavior	Egg-Laying Assay	Egg number [27]
	Two-Choice Mating Trials	Mate preference [36]
	No-Choice Mating Trials	successful mating [36]
Developmental Behavior	Eclosion Percentage	Percentage of adults eclosed from pupa [33,34]

Table 4. Summary of data extraction for research finding's theme

Substance	**Findings**
Cadmium Chloride	Methanolic extracts of AL and AS significantly mitigated behavioral disruptions caused by CdCl2 exposure [29]
Mercuric Chloride	Extracts from ackee leaves and arils improved negative geotaxis and increased offspring emergence and acetylcholinesterase activity [31].
Methylmercury	Exposure to MeHg in male and female flies led to slower climbing, reduced eclosion and flight, and adverse effects on F1 progeny viability, locomotor activity, and sleep patterns [28,32,34].
VCM and VCD	Induced impairments in climbing behavior [37].
Caffeine	Neurons containing the Hugin neuropeptide in the Drosophila larval brain are essential for caffeine avoidance behavior, as their activation results in feeding cessation [39]
Chlordane	Male flies exposed to chlordane (0.1 µM and 0.01 µM) exhibited significant defects in climbing behavior, while female flies remained unaffected [38]
Copper Sulphate	Feeding avoidance of copper showed variations over time and was not notably influenced by strain sensitivity, impacting egg-laying preferences [26]
Copper and Zinc	Flies avoided high concentrations of Cu2+ and Zn2+ ions, impacting egg-laying preferences [27].
Vanadium and Iron	Vanadium exposure led to a decrease in climbing ability [35].
Lead	Sublethal lead exposure during development resulted in altered mate preferences in adult flies [36].
Manganese	Manganese exposure reduced body wall movements and mouth hook movement [24].

Microplastics and Cadmium	Microplastics exposure inhibited locomotor-behavioral function in adult flies [30].
Star Polycation	Larval exposure to star polycation adversely affected climbing ability [25].
Dibutyl Phthalate	DBP disrupted feeding behavior, inducing a hyperphagic response, and affected activity and sleep in Drosophila [42].
Toluene and Formaldehyde	Exposure to toluene or formaldehyde led to significant and varied behavioral alterations in Drosophila [40,41]

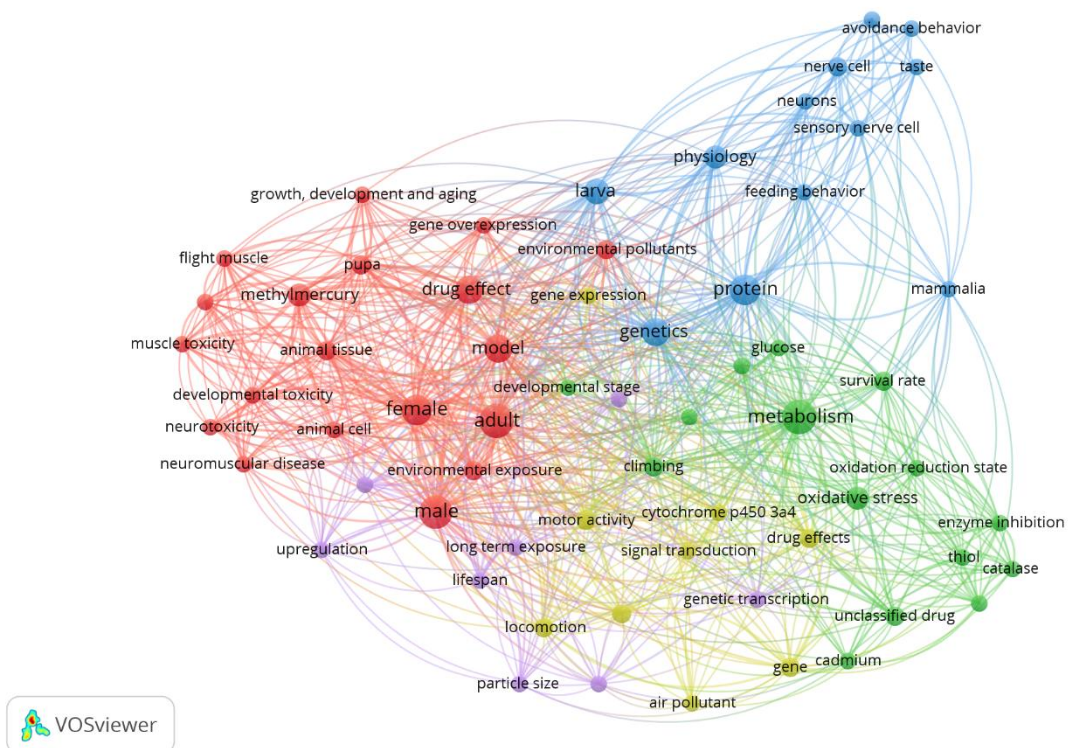

Figure 2. Results of co-occurrence analysis of index keywords

In addition to heavy metals, several other studies have also examined the toxicity of organic compounds, including 4-vinylcyclohexene monoepoxide (VCM) [37], 4-vinylcyclohexene diepoxide (VCD) [37], chlordane [38], caffeine [39], toluene [40,41], formaldehyde [40], and dibutyl phthalate [42]. These organic compounds may originate from industrial processes, pollution, or common consumer products. Testing with Drosophila can reveal the potential toxicity of these organic compounds. Furthermore, the assessment of polymers, especially microplastics [30], reflects global concerns regarding the increasing plastic pollution in the environment [43,44]. Microplastics are small plastic particles that have contaminated aquatic [45] and terrestrial ecosystems [46].

The second theme in this SLR pertains to the variety of behavior assays employed in toxicological studies involving Drosophila. The extraction results for this second theme are presented in Table 3. This theme plays a crucial role in understanding the methodologies employed by different researchers and assessing the comprehensiveness of their toxicological assessments using Drosophila as a model organism. Based on the results of data extraction, various behavioral assays have been used by researchers.

Based on Table 3, various behaviors reported to have been observed in toxicology research are feeding, locomotion, flight, sleep, reproductive, and developmental behavior. Of the various behaviors, feeding behavior showed the most diverse assays [24], [26], [27], [39], [42]. Various data can also be collected, from total consumption [27], [42], food preferences [27], to the number of hook movements in the mouths of Drosophila larvae [24]. These various eating behaviors can provide information regarding Drosophila's ability to deal with foods that contain toxic substances. These assays are also able to reveal changes in nutritional intake and metabolic processes in flies. Much like other organisms, Drosophila exhibits intricate feeding responses influenced by factors like taste, olfaction, nutrient requirements, and circadian rhythms. Researchers frequently customize feeding assays to explore specific dimensions of these behaviors, resulting in a diverse array of tests designed to evaluate various aspects of feeding, including gustatory preferences, olfactory responses, food intake rates, and more.

On the other hand, locomotion behavior is the behavior most often involved by researchers, where climbing behavior is the locomotion behavior most often found in the papers reviewed [25, 2829,30], [32,35,37,38]. Various parameters can be collected from this behavior assay, from the number of flies that successfully climb [25,29,30,35,37,38], movement speed [32,35,38], to the number of body contractions [24]. Through this assay, researchers can study the impact of various toxic compounds on the fly's neuromuscular system. On the other hand, flight assays illuminate the intricacies of an organism's motor functions. Monitoring sleep behavior can uncover disruptions in circadian rhythms or neurological functions, and reproductive behavior assessments highlight toxicity's influence on fertility, mate preferences, and reproductive success. Furthermore, studies of developmental behavior, such as eclosion assays, are pivotal in evaluating long-term effects on growth and metamorphosis. By incorporating these behavioral parameters into toxicological studies using Drosophila, researchers can gain comprehensive insights into the multifaceted impacts of toxicants on an organism's life.

Moreover, the wide array of feeding assays underscores the invaluable role of Drosophila as a model organism in toxicology [24,26,27,39,42]. Furthermore, technological advancements and the emergence of innovative methodologies have broadened the horizons of feeding assays. Researchers can now harness sophisticated imaging and tracking methods, optogenetics, and genetically modified flies to augment the accuracy and comprehensiveness of their analyses of feeding behavior. These methodological breakthroughs have spurred the proliferation of various feeding assay variants, each providing distinct advantages tailored to specific experimental designs.

On the contrary, climbing behavior assays find frequent application in toxicological studies involving Drosophila for several compelling reasons. Firstly, climbing behavior assessments provide a relatively straightforward and robust method for quantifying locomotor performance in these organisms. Evaluating Drosophila's climbing behavior, often measured as the number of flies reaching a specific height within a designated time frame, yields a quantitative and easily interpretable outcome. This simplicity renders climbing assays particularly attractive for toxicological investigations, where the objective often revolves around discerning clear and measurable effects of toxic compounds. Secondly, climbing behavior assays exhibit high sensitivity to a broad spectrum of toxicants, making them versatile tools for toxicity screening. Toxic substances can impact various physiological systems, including neuromuscular function, and these impairments are frequently manifested in the flies' climbing abilities. Consequently, climbing assays can detect subtle toxic effects that might remain unnoticed when using other, less sensitive behavioral assays.

Furthermore, climbing assays are adaptable to high-throughput experimentation, enabling researchers to simultaneously test numerous samples. This scalability proves advantageous when screening extensive libraries of chemicals or conducting dose-response studies to ascertain toxicity thresholds. Moreover, climbing behavior assessments can be conducted without the need for specialized equipment, thereby reducing the overall cost and resource demands associated with toxicological studies. This accessibility encourages a broader spectrum of researchers to incorporate climbing assays into their experimental protocols. Additionally, climbing assays facilitate the assessment of both acute and chronic toxic effects. Researchers can employ short-term assays to evaluate immediate toxicity and conduct longer-term investigations to examine cumulative or delayed impacts on locomotion. This flexibility proves invaluable when scrutinizing the diverse array of toxic compounds and their varied effects on Drosophila behavior.

The final theme extracted in this SLR pertains to the variety of findings from toxicological tests. Some findings indicate negative impacts on Drosophila behavior, such as a decrease in climbing ability, disruptions in feeding behavior, reduced activity, and alterations in sleep patterns (Table 4). Conversely, there are also findings suggesting that certain candidate drugs, such as leaf extracts and ackee arils, can enhance Drosophila behavior, such as improved climbing ability and increased offspring survival. Furthermore, some findings highlight gender-specific responses, as seen in exposure to mercury, which resulted in a decline in climbing ability in both male and female flies.

Behavioral tests, particularly those involving animal models like Drosophila melanogaster, have proven to be robust parameters in the field of toxicology. Behavioral assays enable the observation of an organism's holistic response to a substance. Drosophila often exhibit high sensitivity to harmful substances, even at low exposure levels. This allows for the early detection of potential toxicity of a substance before it reaches levels that could harm humans or the environment. Drosophila behavioral assays can also be conducted relatively quickly and efficiently compared to other toxicological tests that may require more time and resources. This enables faster research on the toxicity of new or potential substances.

In addition to the SLR analysis, this study includes a bibliometric analysis. By using co-occurrence analysis of the index keywords from all the reviewed papers (Figure 2), keywords such as "adult," "male," "metabolism," "female," and "protein" have the highest occurrence values. The frequent appearance of gender-related keywords, such as "male" and "female," in studies examining the toxicity of various substances in Drosophila behaviour assays can be attributed to several factors. First, if we look at humans, gender is an important demographic effect that can influence various aspects, including health aspects, behavioural patterns, and the level of exposure to toxic substances in daily life [47], [48]. Second, if you look at living creatures in general, the metabolic processes and genetic constitution of males and females in many organisms are different, which has the potential to influence the body's response to toxic substances [49], [50]. Differences in the body's response can be caused by differences in hormone levels, detoxification processes, and differences in levels of vulnerability to oxidative stress [51].

Furthermore, the high occurrence value of the adult category can be attributed to the research methods involved in the studies reviewed. The majority of reported behaviour assays involve the imago phase of the fly rather than the larval phase. Then, the high occurrence values of the words metabolism and protein indicate that exposure to toxic compounds is susceptible to changes in the organism's metabolism [52–54]. These metabolic changes may affect gene expression levels in exposed organisms [55]. These changes in metabolism and protein production levels could be the basis underlying changes in various behavioral parameters observed by researchers. In summary, these keywords collectively emphasize the fundamental aspects of toxicological inquiries related to Drosophila behavior assays, facilitating a comprehensive understanding of how toxins impact adult flies, disturb metabolic processes, and modulate protein function. This knowledge not only contributes to our understanding of toxicant-induced behavioral changes in Drosophila but also provides valuable insights that can extend to broader ecological and health-related contexts.

4. CONCLUSION

In this SLR, the identification of three key themes emerges from various studies related to the behavior of Drosophila melanogaster in the context of toxicological assessments. Firstly, a diverse range of substances tested encompasses various types of toxic compounds, from heavy metals to organic compounds, highlighting the versatility of the Drosophila model in evaluating toxicity. Secondly, the variety of behavioral testing methods employed, including feeding, locomotion, flight, sleep, reproductive, and developmental assays, demonstrates the multitude of behavioral dimensions that can be examined in toxicological research. Lastly, there are several intriguing findings related to the effects of various substances on Drosophila behavior, such as alterations in sleep patterns, feeding activity, and climbing ability.

Recommendations stemming from these findings suggest that Drosophila behavior assays should be more widely employed in the context of toxicological assessments. Some substances or compounds have been frequently tested and exhibit clear impacts on fly behavior, making them suitable benchmark parameters for evaluating the toxicity of other substances with less apparent toxic effects. This can facilitate further research in identifying the potential toxic effects of various substances. The SLR results also indicate that there is still room for more in-depth research into the potential therapeutic effects of specific treatments or extracts in mitigating the negative impacts of hazardous substances. This points towards the possibility of developing improved therapies or preventive approaches related to toxicity.

5. REFERENCES

1) Bjørklund G., Dadar M., Mutter J. and Aaseth J. 2017. The toxicology of mercury: Current research and emerging trends. Environmental Research 159: 545-554.
2) Chae Y. and An Y-J. 2018. Current research trends on plastic pollution and ecological impacts on the soil ecosystem: A Review. Environmental Pollution 240: 387-395.
3) Mostafalou S. and Abdollahi M. 2017. Pesticides: an update of human exposure and toxicity. Archives of Toxicology 91(2): 549-599.
4) Damalas C. and Koutroubas S. 2016. Farmers' Exposure to Pesticides: Toxicity Types and Ways of Prevention Toxics 4(1): 1.
5) Patocka J., Wu R., Nepovimova E., Valis M., Wu W. and Kuca K. 2021. Chemistry and toxicology of major bioactive substances in inocybe mushrooms. International Journal of Molecular Sciences 22(4): 2218.
6) Myatt G. J. et al. 2018. In silico toxicology protocols. In silico toxicology protocols 96: 1-17.
7) Sudmeier L.J., Howard S.P. and Ganetzky B. 2015. A Drosophila model to investigate

the neurotoxic side effects of radiation exposure. Disease Models & Mechanisms 8 (7): 669–677.
8) Sood K., Kaur J., Singh H., Arya S.K. and Khatri M. 2019. Comparative toxicity evaluation of graphene oxide (GO) and zinc oxide (ZnO) nanoparticles on Drosophila melanogaster. Toxicology Reports 6: 768-781
9) Zhao M., Kao C.S., Arndt C., Tran D.D., Cho W.I., Maksimovic K., Chen X.X, Khan M., Zhu H., Qiao J. and Peng K. 2020. Knockdown of genes involved in axonal transport enhances the toxicity of human neuromuscular disease-linked MATR3 mutations in Drosophila. FEBS Letters. 594(17): 2800–2818
10) Mirzoyan Z., Sollazzo M., Allocca M., Valenza A.M., Grifoni D. and Bellosta P. 2019. Drosophila melanogaster: a model organism to study cancer. Frontier in Genetic 10: 51.
11) Yamaguchi M. and Yoshida H. 2018. Drosophila as a model organism Drosophila Models for Human Diseases Advances in Experimental Medicine and Biology ed M Yamaguchi (Springer), p. 1–10
12) Fischer F P, Karge R A, Weber Y G, Koch H, Wolking S and Voigt A. 2023. Drosophila melanogaster as a versatile model organism to study genetic epilepsies: An overview. Frontiers in Molecular Neuroscience 16
13) Muha V., Fenckova M., Ferenbach A.T., Catinozzi M., Eidhof I., Storkebaum E., Schenck A. and van Aalten D.M. 2020. O-GlcNAcase contributes to cognitive function in Drosophila. Journal of Biological Chemistry. 295(26): 8636–8646.
14) Cao T., Sujkowski A., Cobb T., Wessells R. J and Jin J-P. 2020. The glutamic acid-rich–long C-terminal extension of troponin T has a critical role in insect muscle functions. Journal of Biological Chemistry 295(12): 3794-3807.
15) Ozaki M., Le T.D. and Inoue Y.H. 2022. Downregulating Mitochondrial DNA polymerase γ in the muscle stimulated autophagy, apoptosis, and muscle aging-related phenotypes in drosophila Adults Biomolecules 12(8): 1105.
16) Choi J.K, Jeon Y.C., Lee D.W., Oh J.M., Lee H.P., Jeong B.H., Carp R.I., Koh Y.H. and Kim Y.S. 2010. A Drosophila model of GSS syndrome suggests defects in active zones are responsible for pathogenesis of GSS syndrome. Human molecular genetics 19(22): 4474–4489.
17) Fan W., Jin X., Xu M., Xi Y., Lu W., Yang X., Guan M.X., Ge W. 2021. FARS2 deficiency in Drosophila reveals the developmental delay and seizure manifested by aberrant mitochondrial tRNA metabolism. Nucleic Acids Research 49: 13108–13121.
18) Ro J., Harvanek Z.M. and Pletcher S.D. 2014. FLIC: High-Throughput, Continuous Analysis of Feeding Behaviors in Drosophila. PLoS One 9: 6
19) Babcock D.T. and Ganetzky B. 2014. An improved method for accurate and rapid measurement of flight performance in Drosophila. Journal of Visualized Experements (84): 51223.
20) Sujkowski A. and Wessells R. 2018. Using Drosophila to Understand Biochemical and Behavioral Responses to Exercise. Exercise and Sport Sciences Reviews 46(2): 112–120.
21) Apostolopoulou A.A., Mazija L., Wüst A. and Thum A.S. 2014. Composition of agarose substrate affects behavioral output of Drosophila larvae. Frontiers in Behavioral Neuroscience 8
22) French A.S., Sellier M.J., Agha M.A., Guigue A., Chabaud M.A., Reeb P.D., Mitra A., Grau Y., Soustelle L. and Marion-Poll F. 2015. Dual mechanism for bitter avoidance in Drosophila. Journal of Neuroscience 35: 9.
23) Khatun S., Mandi M., Rajak P. and Roy S. 2018. Interplay of ROS and behavioral pattern in fluoride exposed Drosophila melanogaster. Chemosphere 209: 220-231.
24) Pankau C., Nadolski J., Tanner H., Cryer C., Di Girolamo J., Haddad C., Lanning M.,

Miller M., Neely D., Wilson R. and Whittinghill B. 2022. Examining the effect of manganese on physiological processes: Invertebrate models Comparative Biochemistry and Physiology 251: 109209.

25) Yan S., Li N., Guo Y., Chen Y., Ji C., Yin M., Shen J. and Zhang J. 2022. Chronic exposure to the star polycation (SPc) nanocarrier in the larval stage adversely impairs life history traits in Drosophila melanogaster. Journal of Nanobiotechnology 20: 515

26) Green L., Coronado-Zamora M, Radío S, Rech G.E., Salces-Ortiz J. and González J. 2022. The genomic basis of copper tolerance in Drosophila is shaped by a complex interplay of regulatory and environmental factors. BMC Biologi 20 1.

27) Xiao S, Baik L.S., Shang X. and Carlson J.R. 2022. Meeting a threat of the Anthropocene: Taste avoidance of metal ions by Drosophila. Proceedings of the National Academy of Sciences of the United States of America. 119(25):e2204238119.

28) Algarve T.D., Assmann C.E., Aigaki T. and da Cruz I.B.M. 2020. Parental and preimaginal exposure to methylmercury disrupts locomotor activity and circadian rhythm of adult Drosophila melanogaster. Drug and Chemical Toxicology 43: 255–265.

29) Ibraheem O *et al.* 2022. Ackee (Blighia sapida K.D. Koenig) leaves and arils methanolic extracts ameliorate cdcl2-induced oxidative stress biomarkers in Drosophila melanogaster. Oxidative Medicine and Cellular Longevity. 2022: 3235031

30) Zhang Y., Wolosker M.B., Zhao Y., Ren H. and Lemos B. 2020. Exposure to microplastics cause gut damage, locomotor dysfunction, epigenetic silencing, and aggravate cadmium (Cd) toxicity in Drosophila. The Science of the Total Environment 744: 140979

31) Ibraheem O., Bankole D., Adedara A., Abolaji A.O., Fatoki T.H., Ajayi J.M. and Eze C.T. 2021. Methanolic Leaves and Arils Extracts of Ackee (Blighia sapida) Plant ameliorate mercuric chloride-induced oxidative stress in Drosophila melanogaster. Biointerface Research in Applied Chemistry 11: 7528-7542.

32) Peppriell A.E., Gunderson J.T., Krout I.N., Vorojeikina D. and Rand M.D. 2021. Latent effects of early-life methylmercury exposure on motor function in Drosophila Neurotoxicology and Teratology 88: 107037

33) Peppriell A.E., Gunderson J.T., Vorojeikina D., Rand M.D. (2020). Methylmercury myotoxicity targets formation of the myotendinous junction. Toxicology 443: 152561.

34) Ohiomokhare S., Olaolorun F., Ladagu A., Olopade F., Howes M.J., Okello E., Olopade J., Chazot P.L. 2020. The Pathopharmacological Interplay between Vanadium and Iron in Parkinson's Disease Models International Journal of Molecular Sciences 21(18): 6719.

35) Peterson E.K., Yukilevich R., Kehlbeck J., LaRue K.M., Ferraiolo K., Hollocher K., Hirsch H.V. and Possidente B. 2017. Integrative behavioral ecotoxicology: bringing together fields to establish new insight to behavioral ecology, toxicology, and conservation Current. Zoology 63 (2): 185–194

36) Abolaji A.O., Kamdem J.P., Lugokenski T.H., Farombi E.O., Souza D.O., da Silva Loreto É.L. and Rocha J.B. 2015. Ovotoxicants 4-vinylcyclohexene 1,2-monoepoxide and 4-vinylcyclohexene diepoxide disrupt redox status and modify different electrophile sensitive target enzymes and genes in Drosophila melanogaster Redox Biology 5: 328–339.

37) Wu Q., Du X., Feng X., Cheng H., Chen Y., Lu C., Wu M. and Tong H. 2021. Chlordane exposure causes developmental delay and metabolic disorders in Drosophila melanogaster Ecotoxicology and Environmental Safety 225: 112739

38) Hückesfeld S., Peters M. and Pankratz M.J.

2016. Central relay of bitter taste to the protocerebrum by peptidergic interneurons in the Drosophila brain Nature Communications 13(7): 12796.

39) Eom H.J., Liu Y., Kwak G.S., Heo M., Song K.S., Chung Y.D., Chon T.S. and Choi J. 2017. Inhalation toxicity of indoor air pollutants in Drosophila melanogaster using integrated transcriptomics and computational behavior analyses. Scientific Reports 7: 1-15.
40) Bushnell P.J., Ward W.O., Morozova T.V., Oshiro W.M., Lin M.T., Judson R.S., Hester S.D., McKee J.M. and Higuchi M. 2017. Editor's highlight: genetic targets of acute toluene inhalation in Drosophila melanogaster. Toxicological Sciences 156: 230–239.
41) Williams M.J., Wiemerslage L., Gohel P., Kheder S., Kothegala L.V. and Schiöth H.B. 2016. Dibutyl phthalate exposure disrupts evolutionarily conserved insulin and glucagon-like signaling in Drosophila males Endocrinology 157(6): 2309–2321
42) Free C.M., Jensen O.P., Mason S.A., Eriksen M., Williamson N.J. and Boldgiv B. 2014. High-levels of microplastic pollution in a large, remote, mountain lake Marine Pollution Bulletin 85(1):156-63.
43) Chia R.W., Lee J-Y., Kim H. and Jang J. 2021. Microplastic pollution in soil and groundwater: a review. Environmental Chemistry Letters 19 (6): 4211 – 4224.
44) Sul J.A.I. and Costa M.F. 2014. The present and future of microplastic pollution in the marine environment Environmental Pollution 185: 352-364.
45) Xu C., Zhang B., Gu C., Shen C., Yin S., Aamir M. and Li F. 2020. Are we underestimating the sources of Microplastic pollution in terrestrial environment? Journal of Hazardous Materials 400: 123228.
46) Wahlang B. 2018. Exposure to persistent organic pollutants: impact on women's health. Reviews on Environmental Health 33(4): 331-348.
47) Arrebola J.P., Fernández M.F., Olea N., Ramos R. and Martin-Olmedo P. 2013. Human Exposure to p,p'-Dichlorodiphenyldichloroethylene (p,p'-DDE) in urban and semi-rural areas in Southeast Spain: A Gender Perspective. Science of The Total Environment 458–460: 209–216.
48) Carneiro M., Gutiérrez-Praena D., Osório H., Vasconcelos V., Carvalho A.P. and Campos A. 2015. Proteomic analysis of anatoxin-a acute toxicity in zebrafish reveals gender specific responses and additional mechanisms of cell stress. Ecotoxicology and Environmental Safety 120: 93-101.
49) Le Manach S., Khenfech N., Huet H., Qiao Q., Duval C., Marie A., Bolbach G., Clodic G., Djediat C., Bernard C. and Edery M. 2016. Gender-Specific Toxicological Effects of Chronic Exposure to Pure Microcystin-LR or Complex Microcystis aeruginosa Extracts on Adult Medaka Fish Environmental Science & Technology. 50: 8324–8334.
50) Kander M.C., Cui Y. and Liu Z. 2017. Gender difference in oxidative stress: a new look at the mechanisms for cardiovascular diseases. Journal of Cellular and Molecular Medicine 21(5): 1024-1032.
51) Mattsson K., Ekvall M.T., Hansson L-A, Linse S., Malmendal A. and Cedervall T. 2015. Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. Environmental Science & Technology. 49(1): 553-61.
52) Lee H.K. and Pak Y.K. 2018. Persistent organic pollutants, mitochondrial dysfunction, and metabolic syndrome. Wiley online library 691–707.
53) Zhang L., Nichols R.G., Correll J., Murray I.A., Tanaka N., Smith P.B., Hubbard T.D., Sebastian A., Albert I., Hatzakis E., Gonzalez F.J., Perdew G.H., Patterson A.D. 2015. Persistent Organic Pollutants Modify Gut Microbiota-Host Metabolic Homeostasis in Mice Through Aryl Hydrocarbon Receptor Activation *Environmental Health Perspectives* 123(7):

679-688.
54) Thomson E.M., Vladisavljevic D., Mohottalage S., Kumarathasan P. and Vincent R. 2013. Mapping acute systemic effects of inhaled particulate matter and ozone: multiorgan gene expression and glucocorticoid activity. Toxicological Sciences 135: 169–181.