Universidade de Aveiro
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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Aplicada, realizada sob a orientação científica da Doutora Maria Pavlaki, Investigadora Doutorada do Departamento de Biologia da Universidade de Aveiro, e da Professora Susana Patrícia Mendes Loureiro, Professora Associada com Agregação do Departamento de Biologia da Universidade de Aveiro.

Dedico este trabalho ao meu avô.

o júri

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agradecimentos

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palavras-chave

Drosophila melanogaster, ecotoxicologia, contaminantes, metais, pesticidas, bisfenóis, fármacos

resumo

Com a constante preocupação devido aos problemas ambientais que vivemos, é cada vez mais importante realizar testes de toxicidade com relevância ecológica. Neste contexto, a utilização de organismos modelo para avaliar desafios ambientais tem vindo a aumentar, especialmente o uso de *Drosophila melanogaster*, que emergiu como um modelo económico e preponderante oferecendo informações valiosas sobre as respostas ecológicas e genéticas após a exposição a vários contaminantes.

Este trabalho engloba uma revisão sistemática da literatura sobre a aplicação de *D. melanogaster* em testes ecotoxicológicos. A revisão contém estudos focados no impacto de poluentes como metais, pesticidas, bisfenóis e fármacos na *Drosophila*. Foram analisadas as respostas da mosca da fruta a estes fatores de stress ambiental, recorrendo a parâmetros comportamentais, reprodutivos, fisiológicos e bioquímicos. Os resultados destacaram a elevada toxicidade da maioria das substâncias testadas, revelando a necessidade de realizar estudos adicionais que considerem cenários ecológicos reais, com interações dinâmicas entre as diversas espécies da cadeia alimentar e os efeitos de uma exposição prolongada. Adicionalmente, destaca a importância de investigar os mecanismos moleculares e genéticos subjacentes aos efeitos observados além do potencial de transposição para outros organismos, de forma a melhorar a avaliação das implicações ecológicas e na saúde humana.

Assim, esta dissertação sublinha o papel da *D. melanogaster* como uma ferramenta fundamental para a avaliação ecotoxicológica, bem como a possível integração em pesquisas futuras que conduzam à aplicação de estratégias melhoradas na atenuação da poluição dos ecossistemas.

keywords *Drosophila melanogaster*, ecotoxicology, contaminants, metals, pesticides, bisphenols, pharmaceuticals

abstract

With the constant concern over the environmental problems we are experiencing, it is becoming progressively more important to conduct ecologically relevant toxicity tests. In this context, the use of model organisms to assess environmental challenges has been increasing, especially the use of *Drosophila melanogaster*, which has emerged as an economical and preponderant model offering valuable information on the ecological and genetic responses after exposure to various contaminants.

This work includes a systematic review of the literature on the use of *D. melanogaster* in ecotoxicological tests. The review contains studies focusing on the impact of pollutants such as metals, pesticides, bisphenols, and pharmaceuticals on *Drosophila*. The fruit fly responses to these environmental stressors were analysed using behavioural, reproductive, physiological, and biochemical endpoints. The results highlighted the high toxicity of most substances assessed, revealing the need to conduct additional studies that consider natural settings, with dynamic interactions between the various species in the food chain and the effects of prolonged exposure. In addition, it highlights the importance of investigating the molecular and genetic mechanisms underlying the effects observed, as well as the potential for transposition to other organisms, which can improve the assessment of ecological and human health implications.

Thus, this dissertation highlights the role of *D. melanogaster* as a fundamental tool for ecotoxicological evaluation, as well as its integration into future research leading to the application of improved strategies for the mitigation of ecosystem pollution.

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Chapter I: General Introduction

Contextualisation

The countless practical and ethical obstacles of using humans in laboratory experiments created the urge of studying biological processes using rats and mice as model organisms (Jennings, 2011). These species, among others, have provided important developments in fundamental and applied Biology, Biochemistry and Biomedicine (Morimoto & Pietras, 2020). Moreover, through the years, invertebrates have increasingly contributed to scientific progress in several areas such as genetics, ecology, and physiology (Demir, 2020). One of the most studied model organism is the 2-3 mm arthropod *Drosophila melanogaster*, from the order Diptera and the family Drosophilidae, considered an ecological generalist due to its broad distribution, which explains why it is so easily propagated in a laboratory setting (Demir, 2020; Markow, 2015). The relevance of this specimen is directly linked to the assessment of developmental and cellular processes that are shared by various other organisms, including humans (Demir, 2020; Marques et al., 2018).

The *D. melanogaster* life cycle, represented in Figure 1 includes four stages of development - embryo, larva, pupa, and adult – and normally takes between 10 to 12 days at 25 °C to complete the process (Anushree, Ali, Bilgrami, et al., 2023; Rand, 2010). At this temperature the embryo evolves into the first instar larva in 24 hours, one day after it transforms into the second instar larva that changes into the third instar larva the following day. This bigger larva develops into a pupa which takes about 2 to 3 days. Finally, the pupal stage lasts about 5 to 6 days (Markow, 2015; Rand, 2010), resulting in a newly hatched adult completing the process called metamorphosis.

Females are considered fertile for about 20 days and lay approximately 100 eggs per day. Despite, the flies' cultures can be preserved at 18 °C, which slows down the life cycle and makes it easier to maintain them between experiments (Jennings, 2011).

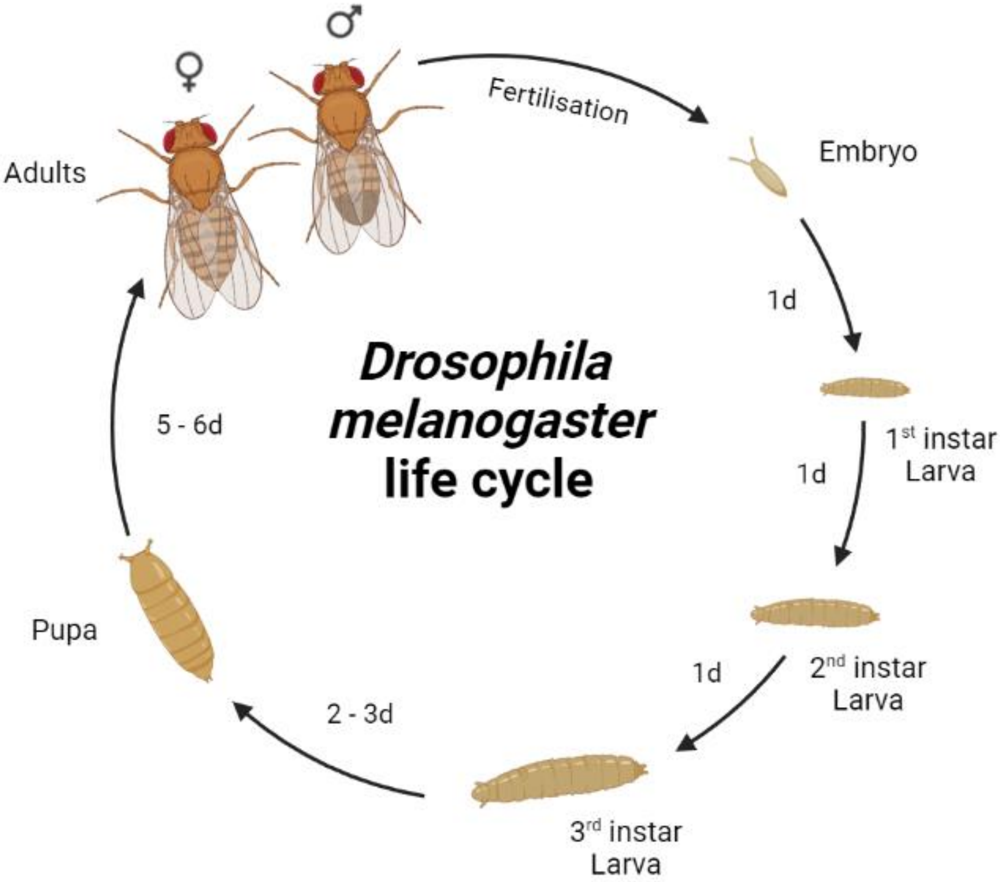

Figure 1 - The life cycle of *Drosophila melanogaster*. Created with BioRender.com.

In the wild, this fly is found in rotten fruits and vegetables and feeds on the microorganisms that grow in these conditions (Jennings, 2011). Females lay their eggs in decomposing matter and the following two life stages develop there. The embryos and the pupae are completely immobile which can lead to adaptations to survive under different environmental pressures (Markow, 2015).

In the laboratory context, *D. melanogaster* has been used as a model organism for over a century and since then has become indispensable for toxicity and genetic analyses given that researchers can use embryonic, larval, pupal, and adult stages (Etuh et al., 2021; Jennings, 2011). They breed the flies at constant temperature and humidity in small flasks of 20 to 50 mL with a solid standard medium usually composed by distilled water, agar-agar, sucrose, sodium chloride and yeast (Ferreira et al., 2019; Marques et al., 2018) treated with propionic acid to avoid mould formation (Markow, 2015; Rand, 2010) and closed with cotton wool. The flies are anesthetized with carbon dioxide or ether and moved with a little brush whenever is needed to manipulate and view them through a stereomicroscope or a magnifying glass (Jennings, 2011).

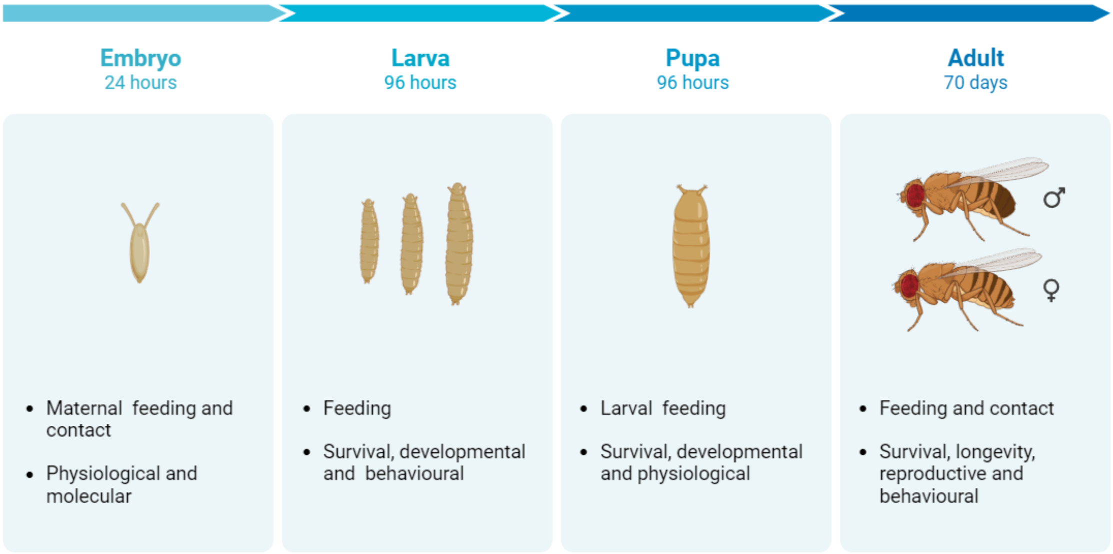

Figure 2 - Toxicological endpoints sorted by *D. melanogaster* stages of development. The usual exposure times are presented below the development stages. Adapted from Rand et al. (2023). Created with BioRender.com.

In toxicology, a variety of endpoints is readily tested depending on the life stage (Rand et al., 2023). In Figure 2, the most common endpoints are arranged by developmental stages, including the different exposure routes and the usual test duration. The first life stage has the lowest amount of tested endpoints as a result of limited consistent dosing (Rand et al., 2023). The following two stages – larva and pupa – have important roles in developmental and survival testing while the assessment of behavioural changes is only possible in adults and larvae due to its motile function. Nonetheless, adults are the most used in survival, longevity and reproductive assays since its very convenient for feeding and controlling the concentration of chemicals (Rand et al., 2023).

Furthermore, fruit flies require minimal maintenance, have a short life cycle and are easy to manipulate which allows having large samples at a relatively low price (Carlson et al., 2008; Chifiriuc et al., 2016; Makos et al., 2009). These experimental qualities and the lack of ethical and safety issues (Demir, 2020) make *D. melanogaster* an ideal model organism for different types of assays, which replaces vertebrates in this important function. The fact that this was one of the first species with a complete genome sequencing also plays a role in choosing this species as foundation for environmental and human health studies (Makos et al., 2009).

Use of *Drosophila melanogaster* in human toxicology

The use of *D. melanogaster* as a model organism for the research of various human diseases is very common, since there are many signal pathways and conserved genes that are homologous to the fruit fly (Affleck et al., 2006; Carlson et al., 2008), including the Wnt, TGFβ, Hedgehog, EGF, cytokine and Notch pathways (Rand, 2010). More precisely, the genome is approximately 60% identical, while 75% of genes related to human diseases have homologs in this invertebrate (Demir, 2020; Vecchio, 2015), in some cases sharing more than 90% of the nucleotide sequence (Chifiriuc et al., 2016). Human disorders like epilepsy, schizophrenia, autism spectrum disorders (ASD), and Tourette syndrome are determined by the orthologs CASK, NLGN4, and NLGN2 (Welch et al., 2022). With this growing knowledge, Drosophila studies can present new means for preventing, supervising and treating such disorders (Mohr & Perrimon, 2019).

For example, this invertebrate has been used in studies that emphasize on the neurotoxic effects of heavy metals such as arsenic (Anushree, Ali, Bilgrami, et al., 2023; Anushree, Ali, & Ahsan, 2023). In Anushree, Ali, Bilgrami, et al. (2023), development, oxidative stress, neurotransmitter, and behavioural assays were performed for assessing the negative impacts of arsenic on *D. melanogaster*. Acute exposure to arsenic significantly delayed pupal development, and decreased the climbing ability, indicating neurological impairments. This study also showed high levels of oxidative stress markers, and altered levels of neurotransmitters, including dopamine and serotonin, indicating potential disruption in the nervous system (Anushree, Ali, Bilgrami, et al., 2023).

Additionally, *D. melanogaster* can be used as an instrument to estimate in what way chemical contaminants are capable of inducing the development of Parkinson-like disease (Musachio et al., 2020). In this study, the motor functions of exposed flies were evaluated through climbing assays and movement tracking, neurodegenerative and oxidative stress analysis were also performed. *Drosophila* exposed to BPA exhibited reduced climbing ability and locomotor deficits similar of the motor symptoms seen in humans with Parkinson´s disease. Moreover, BPA exposure resulted in the degeneration of dopaminergic neurons in the flies' brain, being also an indicator of Parkinson's disease (Musachio et al., 2020). These findings suggest how the constant exposure to these chemicals can alter the adult human brain.

Hence, most common applications include the study of metabolism and its association with ageing, illnesses like cancer (Ferreira et al., 2019), autism, diabetes and mechanisms of immunity and degenerative disorders (Demir, 2020), using for example the somatic mutation and recombination test (SMART) or wing spot test (Marques et al., 2018), and the negative geotaxis method or climbing assay (Carlson et al., 2008) as methodologies for translating these types of biological changes to other organisms, specially humans.

Use of *Drosophila melanogaster* in ecotoxicology

Chemicals like heavy metals, pesticides, nanomaterials, and other types of toxicants are incredibly widespread and intensively used in many human activities, resulting in high concentrations in different ecosystems (Demir, 2020; Frat et al., 2021; Vecchio, 2015). Both natural and experimental strains of *Drosophila* have been explored into reporting and understanding their responses to changes in the environment (Markow, 2015).

Thus, in addition to all the applications in human health this organism has also shown to have a promising future in the field of ecotoxicology. Being one of the best well-known eukaryotes (Demir, 2020), *D. melanogaster* can provide information on the evaluation of contaminant's impact on the environment at a biological organizational level, from cells to ecosystems (Frat et al., 2021). Toxicological assays can be performed in every stage of *D. melanogaster*, allowing to examine the effect that these products have on the behaviour and development of this organism (Chifiriuc et al., 2016), as seen in Figure 2.

Things like the constant emergence of new chemicals, the pesticide resistance in several insects and the intense usage of heavy metal in daily human activities are propelling these types of research (Frat et al., 2021; Markow, 2015; Rand, 2010). Moreover, currently due to the advancements in technology is very easy to examine the smaller details in the tissues of all developing life stages and behaviours like courtship, mating, locomotion, and fecundity represent a relevant tool for this area (Rand, 2010).

Aims of the present work

The purpose of this dissertation is to study the relevance of using *Drosophila melanogaster* as a model organism in ecotoxicological research by assessing its responses to environmental contaminants. Therefore, examining the ecological and toxicological implications of chemical exposure on different life stages, including its developmental, reproductive, and behavioural responses.

As a result, this work has the potential to expand our understanding of the environmental consequences of human activities and chemical pollutants by utilizing this invertebrate as a valuable tool for environmental risk assessment and the development of ecologically relevant toxicity assays. Ultimately, contributing to the advancement of ecotoxicology by establishing *D. melanogaster* as a versatile and cost-effective model for assessing the effects of contaminants on both individual organisms and entire ecosystems.

Dissertation Structure

The present dissertation is organized in three chapters.

In "Chapter 1: General Introduction", *Drosophila melanogaster* is advocated as a fundamental model organism in scientific investigation due to its rapid development and genetic homology with humans. Its short life cycle, ease of maintenance, and ethical benefits make it indispensable for toxicology studies. *Drosophila* presents a great advantage in understanding human diseases, and serves as a powerful tool for ecotoxicology, examining the environmental impact of contaminants. Its behavioural, genetic, and reproductive responses provide critical views for both human health and ecotoxicological research.

In "Chapter 2: A Systematic Review in the Use of *Drosophila melanogaster* in Ecotoxicological Tests", a comprehensive search report of studies is carried out with *D. melanogaster* as a model organism for ecotoxicological tests, to achieve a complete picture of the risk of exposure to different types of chemicals. By systematically synthesizing and analysing the available literature, making a wide-range evaluation of different parameters that include *Drosophila* endpoints, most common tests, routes of exposure and different categories of chemicals, this review presents valuable insights for better understanding the broader ecological and health implications of environmental contaminants.

In "Chapter 3: Final Considerations and Future Perspectives", *Drosophila melanogaster* is confirmed as a valuable model organism for assessing chemicals impacts. The future research should focus on bridging the gap between laboratory findings and real-world ecological contexts, exploring multi-species interactions, and understanding the molecular and genetic mechanisms underlying observed effects. Collaborations across investigation fields could also enhance our understanding of ecological and genetic responses to contaminants, potentially leading to more effective regulatory measures.

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Chapter II: A Systematic Review in the Use of *Drosophila melanogaster* in Ecotoxicological Tests

1. Introduction

The evaluation of the environmental impact of contaminants, including metals, pesticides, pharmaceuticals, and industrial chemicals like solvents, surfactants, paints, and polymers, is a critical concern in today's world. As industries expand and agriculture intensifies, the release of chemical compounds continues to rise, posing a significant threat to both terrestrial and aquatic environments (Peterson & Long, 2018).

During the past decades, the scientific community and regulatory agencies have become progressively more aware of the implications of pollutants on ecosystems (Bickham et al., 2000), particularly since the majority of these substances are not tested beforehand (Peterson & Long, 2018).

Contaminants like heavy metals and pesticides are immensely widespread, which obviously has an impact on the whole ecosystem (Gui & Grant, 2008) and affects the populations on multiple levels (Klerks et al., 2011).

Even though the loss of biodiversity is determined by various factors, the effects of acute and sublethal or chronic exposure to a range of contaminants, is proved to be a source of individual’s decline (Bickham et al., 2000; Peterson & Long, 2018). When it interferes with survival and reproduction, those with less sensibility to the pollutant will most likely be favoured by natural selection (Klerks et al., 2011). For example, an organism exposed to a toxic metal or pesticide activates a number of physiological responses that ultimately can culminate, depending on the duration and intensity of exposure, in complications on the reproductive and neurological systems, further severe health problems or even mortality (Chen et al., 2023; Dallinger & Höckner, 2013; Krüger et al., 2021).

To understand the ecological and health risks associated with these contaminants, ecotoxicological research applies a variety of model organisms that serve as substitutes for the broader biological world, determining the toxic effects on molecular, cellular, and/or physiological functions (Dallinger & Höckner, 2013). Therefore, the major objective of ecotoxicology is to measure the impact of chemicals in the natural environment through the assessment of acute and chronic exposure to those chemicals and their respective effects (Peterson & Long, 2018).

As a consequence of increasing ethical questions to animal testing it was necessary to incorporate new methods without sacrificing vertebrates in laboratory tests (Peterson & Long, 2018). Hence, invertebrates pose a very promising alternative to eco- and toxicologic testing. Among these model organisms, *Drosophila melanogaster*, commonly known as the fruit fly, has emerged as a powerful and versatile tool in ecotoxicological research. Its rapid lifecycle, well-characterized genetics, and ability to be easily cultured in the laboratory have made it an ideal candidate for investigating the toxicological effects of various environmental stressors (Peterson & Long, 2018).

D. melanogaster has been widely employed in studies assessing the impacts of metals, pesticides, and plastics on living organisms and ecosystems. For instance, *Drosophila*'s midgut is equivalent to the gastrointestinal tract of mammals therefore resembles vertebrates at anatomic, biochemical and physiological levels (Chen et al., 2023).

In the following sections, the findings and methodologies of studies that have utilized *D. melanogaster* to assess the effects of metals, pesticides, pharmaceuticals, and industrial chemicals like bisphenols will be analysed. This will provide a comprehensive run down of tests assessed, contaminants used, exposure pathways, and endpoints measured. By systematically evaluating the available evidence, the intent is to contribute to the growing supply of knowledge that guides environmental policy, risk assessment, and pollution management strategies. Hence, seek to improve our understanding of the intricate relationship between contaminants and the natural world, promoting a more informed and proactive approach to the protection of our ecosystems.

More precisely, the purpose of this review is to determine the suitability of *Drosophila melanogaster* as a model organism for ecotoxicological tests, considering its relevance, advantages, and limitations in this context. Likewise, explore the insights gained from the use of this organism in ecotoxicological tests, both in terms of its contributions to ecological understanding, and its potential implications for genetic and molecular research in vertebrates. Based on this, the review also aims to find any deficit of information and the limitations of using the fruit fly as an experimental model in ecotoxicology, to enhance our understanding of the ecological and genetic responses of *D. melanogaster* to environmental contaminants. Lastly, this work hopes to provide recommendations for future research, including strategies to improve methodologies, and even promote new areas of study that can lead to significant contributions for ecotoxicological studies.

2. Materials and Methods

This review followed the guidelines from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews (PRISMA-ScR). A comprehensive search was executed in Web of Science and Scopus databases, using the following algorithm: drosophila AND ecotoxicology; drosophila (as Article title, Abstract and Keywords) AND ecotoxicology (as Article title, Abstract and Keywords), respectively. The search retrieved 196 articles, 118 from Web of Science and 78 from Scopus. After a preliminary scan, 42 duplicates were found, leaving the number of articles to 154, that were subjected to a selection by title, abstract, and keywords depending on the inclusion/exclusion criteria presented below.

During this process of title and abstract screening, only research articles published in English were considered. Other publications such as reviews, books and conference papers were not included. Merely studies that utilised *Drosophila melanogaster* as the main tool for risk assessment of pollutants were included. Additionally, only studies concerning the exposure of *D. melanogaster* to at least one of the following contaminants - metals, pesticides, pharmaceuticals, and bisphenols (representing industrial chemicals) - were included in the final selection. Afterwards, the set of data from these articles was transferred into an Excel spreadsheet (Supplementary Material). The information retrieved was sorted and categorized according to: i) contaminants used; ii) exposure routes; iii) type of tests performed; iv) endpoints measured.

3. Results

The number of studies in each segment of the literature search and selection is illustrated in Figure 3. After the title, abstract, and keywords screening of 154 articles, a total of 56 articles were identified as relevant and were put through full text analyses. Twenty-nine articles were excluded due to: studies with genomic evaluation only; contaminants belonging to other categories of chemicals, for example: phytochemicals, acrylamides, plant and fungal volatile compounds, and radiation. The final selection resulted in 27 articles as focus of this review that comply to the selection criteria.

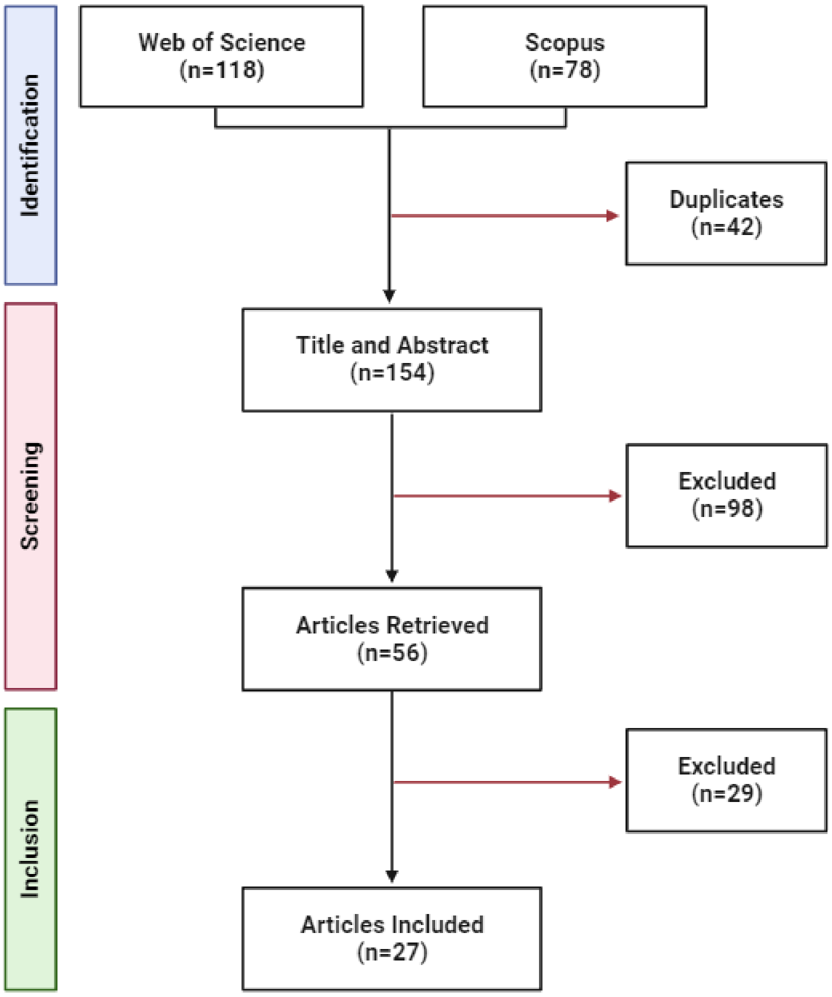

Figure 3 - Flow diagram of the selection process. n=number of articles. Created with BioRender.com.

The selected articles were published between 2009 and 2023. Almost 75% of the articles were published in the last ten years and more than 55% issued between 2019 and 2023 (Figure 4).

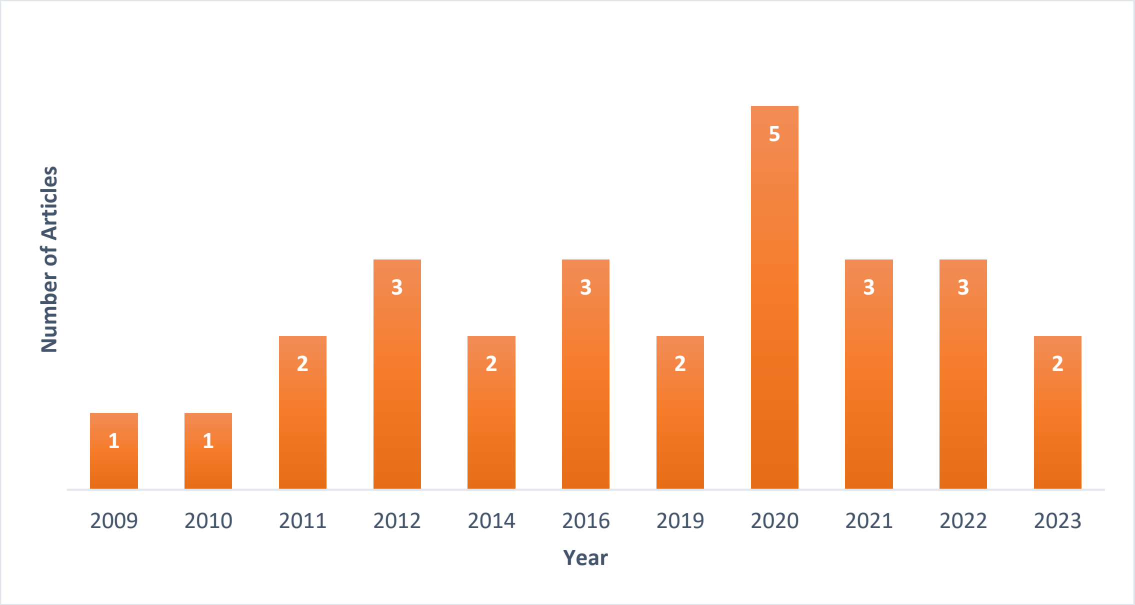

Figure 4 - Number of selected articles by year of publication.

i. Type of Contaminants

According to the selection criteria defined initially, the contaminants were classified based on their origin and respective properties, resulting in 5 categories – Bisphenols, Metals, Pesticides, Pharmaceuticals, and a Combined group that illustrated the publications with more than one category of chemicals tested. The 27 studies were therefore distributed to these groups (Figure 5). The most studied group was Metals, with 48% of the performed studies, particularly cadmium (Cd) (Hu et al., 2019; Sun et al., 2022); mercury (Hg) (Paula et al., 2012); as well as the exposure to both mercury and cadmium (Frat et al., 2021; Saini et al., 2020). Other metals included cerium (Ce) (Huang et al., 2009; B. Wu et al., 2012); copper (Cu) (Pölkki & Rantala, 2020); and a side by side comparison between copper and copper oxide nanoparticles (CuO NPs) (Budiyanti et al., 2022). Finally, the last metallic nanoparticles tested were zinc oxide nanoparticles (ZnO NPs) (Anand et al., 2016); silver nanoparticles (Ag NPs) (Panacek et al., 2011); titanium dioxide nanoparticles (TiO_2 NPs) (Cvetković et al., 2020); and a comparative testing of the last two (Posgai et al., 2011).

Pesticides was the group that followed, representing 30% of the articles. This included chlordane (Q. Wu et al., 2021); chlorpyrifos (Gupta et al., 2010); cryolite (Haller et al., 2016); atrazine (Marcus & Fiumera, 2016; Vogel et al., 2014); exposure to glyphosate, Roundup®, and polyethoxylated tallow amine (POEA) (Bednářová et al., 2020); mixed exposure to cypermethrin, etofenprox, and permethrin (Schleier & Peterson, 2012); and a comparative experiment with Spectracide® and pure atrazine (Chaudhuri et al., 2020).

The group of Bisphenols completed 11%, including bisphenol A (BPA) (Chen et al., 2022; Weiner et al., 2014); and the exposure to bisphenol A along with its derivates (Wang et al., 2023).

Pharmaceuticals were only tested in one article using ciprofloxacin (Liu et al., 2019).

The Combined category comprised of two publications, the assessment of bisphenol A toxicity combined with the effects of cerium oxide nanoparticles (CeO NPs) (Sarkar et al., 2021); and a comparative evaluation of glyphosate, bisphenol A, mercury and cadmium (Frat et al., 2023).

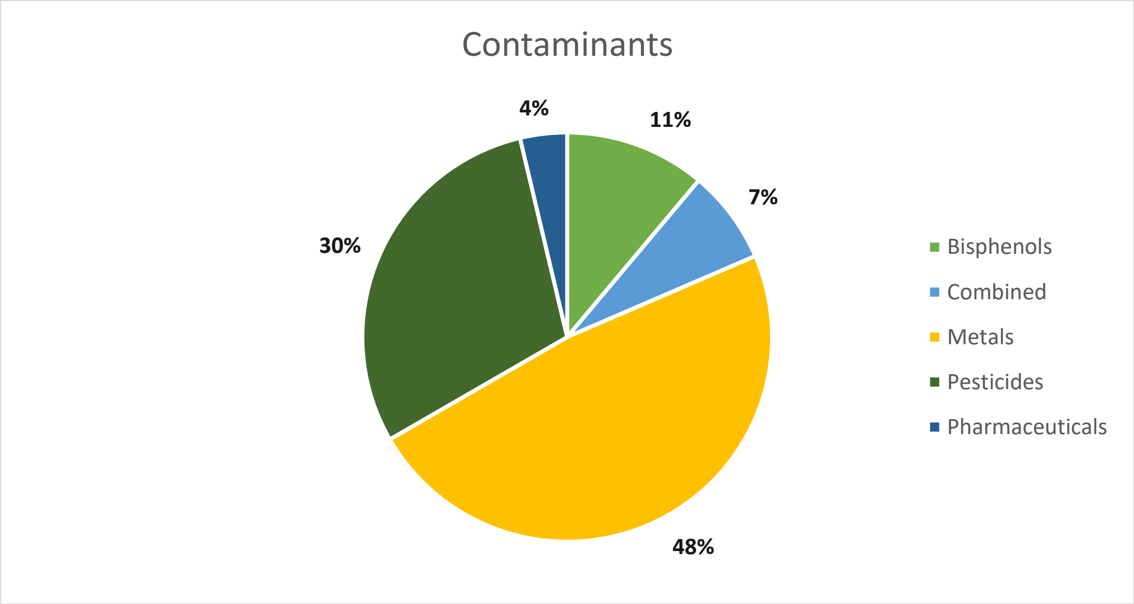

Figure 5 - Categorisation of selected articles according to group of environmental contaminants tested.

ii. Type of Exposure Routes

Regarding the exposure routes (Figure 6), 85% of studies employed the Oral exposure route to expose *D. melanogaster* to bisphenols (Chen et al., 2022; Sarkar et al., 2021; Wang et al., 2023; Weiner et al., 2014) metals (Anand et al., 2016; Budiyanti et al., 2022; Cvetković et al., 2020; Hu et al., 2019; Huang et al., 2009; Panacek et al., 2011; Paula et al., 2012; Pölkki & Rantala, 2020; Posgai et al., 2011; Saini et al., 2020; Sun et al., 2022; B. Wu et al., 2012); pesticides (Chaudhuri et al., 2020; Gupta et al., 2010; Haller et al., 2016; Marcus & Fiumera, 2016; Vogel et al., 2014; Q. Wu et al., 2021); and pharmaceuticals (Liu et al., 2019).

Only one article, resulting in 4% of all articles, used exposure by contact exclusively to test the joint toxicity of insecticides (Schleier & Peterson, 2012).

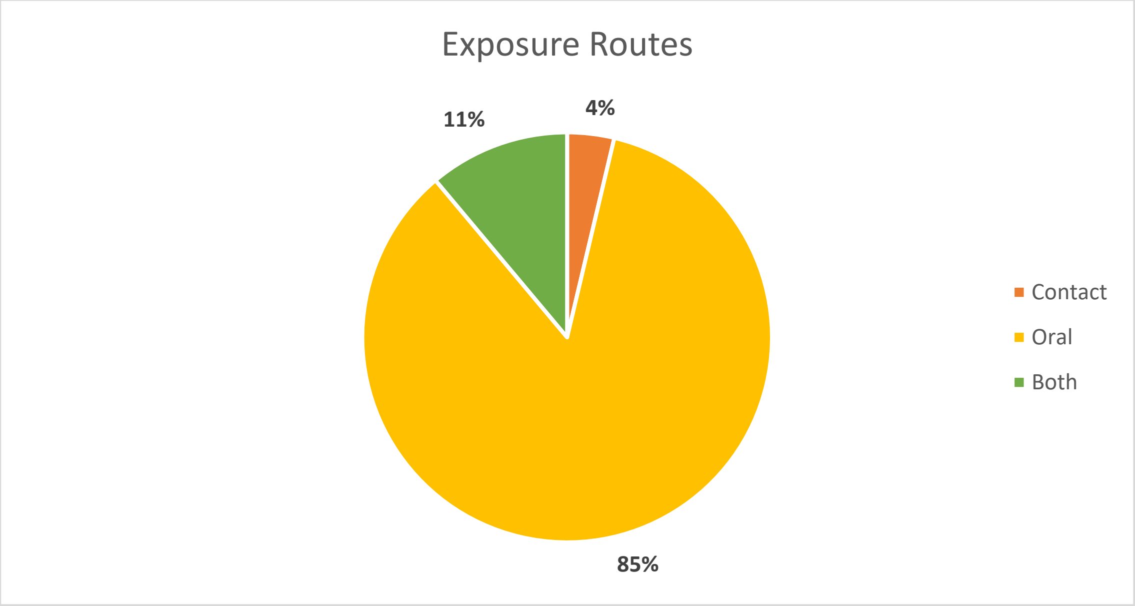

Figure 6 - Articles categorised by exposure route.

Lastly, three studies elected both exposure routes, representing the remaining 11%, to assess the toxicity of metals (Frat et al., 2021); pesticides (Bednářová et al., 2020); and a combination of different pollutants (Frat et al., 2023).

iii. Type of Assays

In Figure 7, the publications are divided by acute and chronic tests, and a third category that involves both tests. The most used was purely Acute toxicity testing, totalizing almost 60% of the experiments, that includes exposure to bisphenols (Wang et al., 2023; Weiner et al., 2014); metals (Hu et al., 2019; Paula et al., 2012; Posgai et al., 2011; Saini et al., 2020; Sun et al., 2022; B. Wu et al., 2012); pesticides (Chaudhuri et al., 2020; Gupta et al., 2010; Haller et al., 2016; Schleier & Peterson, 2012; Vogel et al., 2014; Q. Wu et al., 2021);

pharmaceuticals (Liu et al., 2019); and a mixture of pesticides, bisphenols and metals (Frat et al., 2023).

A total of 22% of the publications performed both tests, essentially with metals (Budiyanti et al., 2022; Frat et al., 2021; Panacek et al., 2011; Pölkki & Rantala, 2020); but also with pesticides (Bednářová et al., 2020) and bisphenols (Sarkar et al., 2021).

The Chronic toxicity testing alone was done in 5 studies, including the exposure to atrazine (Marcus & Fiumera, 2016), bisphenols (Chen et al., 2022), and different metals (Anand et al., 2016; Cvetković et al., 2020; Huang et al., 2009).

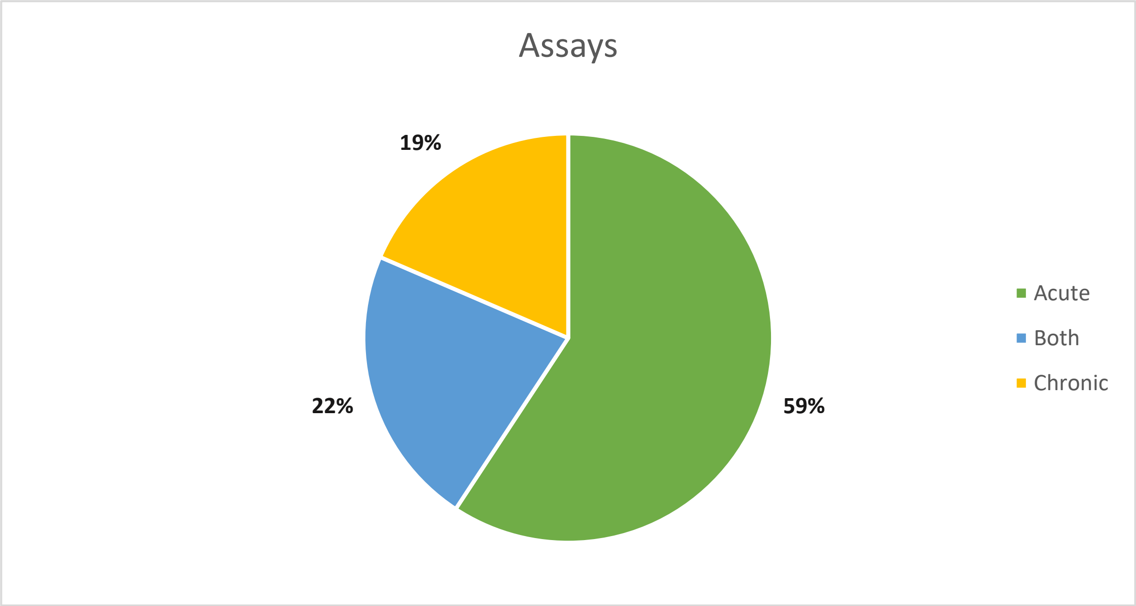

Figure 7 - Categorisation of selected articles by type of test used.

iv. Type of Endpoints

Unlike the sections above, the number of measured endpoints were counted in relation to the number of experiments carried out in each article. In this case, from the 27 articles, 67 experiments were retrieved. The measured endpoints chosen by the studies assessed in this review were divided into the six categories demonstrated in Figure 8.

The most tested endpoint was Development, having a total of 17 experiments. The Development endpoint included parameters such as body and wing size (Cvetković et al., 2020; Haller et al., 2016; Sun et al., 2022; Q. Wu et al., 2021), weight (Marcus & Fiumera, 2016; Weiner et al., 2014), and development between life stages (Budiyanti et al., 2022; Frat et al., 2021, 2023; Liu et al., 2019; Panacek et al., 2011; Pölkki & Rantala, 2020; Posgai et al., 2011; Sarkar et al., 2021; Vogel et al., 2014; Wang et al., 2023; B. Wu et al., 2012).

The next most popular endpoints, with 14 assays, include Survival assays (Budiyanti et al., 2022; Frat et al., 2023; Haller et al., 2016; Panacek et al., 2011; Paula et al., 2012; Posgai et al., 2011; Schleier & Peterson, 2012; Wang et al., 2023) and Longevity analysis (Anand et al., 2016; Bednářová et al., 2020; Huang et al., 2009; Liu et al., 2019; Marcus & Fiumera, 2016; Sarkar et al., 2021), being considered as crucial endpoints for assessing acute and chronic exposure to pollutants.

Likewise, in 14 experiments different Biochemical endpoints were tested through the evaluation of parameters like oxidative stress (Anand et al., 2016; Bednářová et al., 2020; Chaudhuri et al., 2020; Frat et al., 2023; Gupta et al., 2010; Huang et al., 2009; Liu et al., 2019; Saini et al., 2020; Q. Wu et al., 2021) and enzyme activity (Chen et al., 2022; Hu et al., 2019; Paula et al., 2012; Posgai et al., 2011; Sarkar et al., 2021).

These three endpoint categories compose more than 65% of the total number of trials.

The fourth and fifth most used endpoints were Reproductive and Behavioural analyses, both with 8 assays each. This incorporated factors such as fertility (Budiyanti et al., 2022; Huang et al., 2009; Marcus & Fiumera, 2016; Posgai et al., 2011; Vogel et al., 2014) and fecundity (Bednářová et al., 2020; Hu et al., 2019; Pölkki & Rantala, 2020). Behavioural changes included climbing, crawling and movement in general (Anand et al., 2016; Budiyanti et al., 2022; Chaudhuri et al., 2020; Chen et al., 2022; Paula et al., 2012; Sarkar et al., 2021; Wang et al., 2023; Q. Wu et al., 2021).

Lastly, 6 experiments used Genetic endpoints that showed DNA changes in *D. melanogaster* (Anand et al., 2016; Frat et al., 2021; Gupta et al., 2010; Hu et al., 2019; Sun et al., 2022; B. Wu et al., 2012).

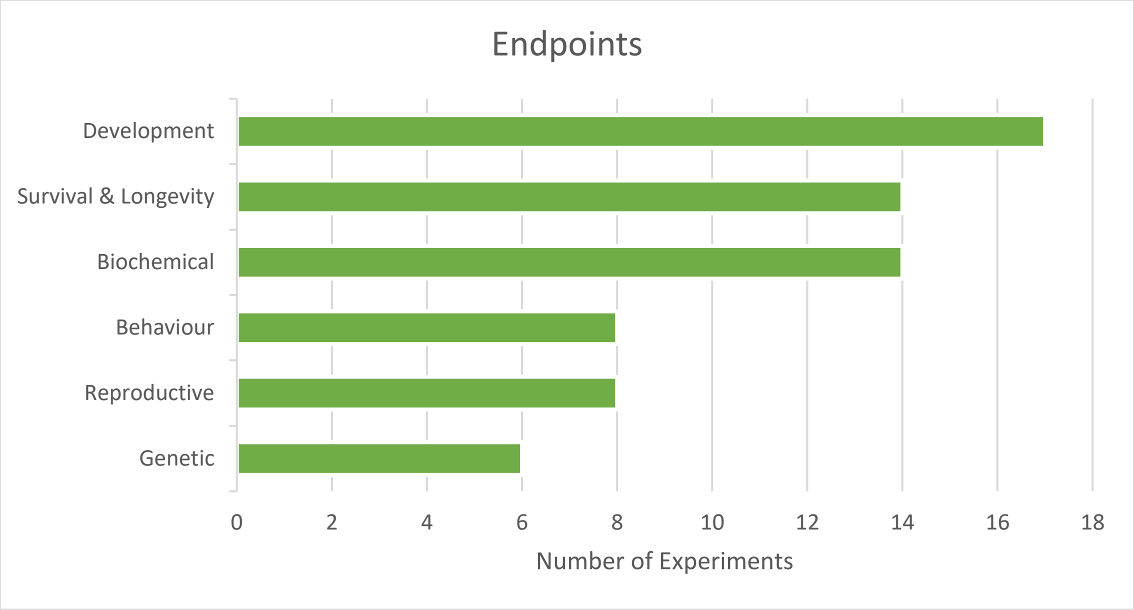

Figure 8 - Number of experiments employing the different type of endpoints by category.

4. Discussion

This review aimed to determine the true potential of the model organism *Drosophila melanogaster* as a valuable tool for ecotoxicological tests, specifically by assessing the acute and chronic exposure to bisphenols, metals, pesticides, and pharmaceuticals as well as combinations of these pollutants.

In terms of toxic effects, survival rates and *Drosophila* life span decreased in most studies independent of the contaminant tested. For example, chronic exposure to 0.5 mM of BPA led to a significant reduction in the survival rate of adult flies after 7 days (Chen et al., 2022). In Wang et al. (2023) other bisphenols were examined, with bisphenol Z (BPZ) being the most toxic and bisphenol E (BPE) being the least toxic. The exposure to ciprofloxacin significantly reduced the longevity of both male and female flies, and the lethal concentrations (LC_{50}) decreased with increasing of the time of exposure (Liu et al., 2019).

Acute exposure to sub-lethal concentrations of Roundup® and POEA reduced the life-span of females *D. melanogaster*, while pure glyphosate did not significantly affect longevity (Bednářová et al., 2020; Frat et al., 2023). This suggests that the formulated herbicide and surfactant can be the cause of the reduced lifespan (Bednářová et al., 2020). In Schleier & Peterson (2012), the most toxic pyrethroid pesticide was cypermethrin, followed by etofenprox and permethrin. They also showed that some mixtures had additive effects, while others exhibited synergistic and antagonistic interactions, depending on their respective concentrations (Schleier & Peterson, 2012). Additionally, cryolite and atrazine exposure had significant effects on *D. melanogaster* like reduced survival and life span in higher concentrations (Haller et al., 2016; Marcus & Fiumera, 2016). Frat et al. (2023) seem to agree with the previous study, where the highest concentrations of atrazine and cryolite caused larval mortality. Exposure to different concentrations of chlordane resulted in dose-dependent mortality and the higher concentrations led to almost complete mortality, particularly during the larval and pupal stages (Q. Wu et al., 2021). In studies with *Danio rerio*, sub-lethal atrazine exposure also significantly affected the mechanisms of oxidative stress (Blahová et al., 2013). Interestingly, flies exposed to 20 ppm of atrazine had longer lifespans than those exposed to intermediate concentrations (Marcus & Fiumera, 2016).

At low concentrations of Cd and Hg, there were no significant effects on the survival of *Drosophila* larvae (Frat et al., 2021), and even at the highest tested cerium concentration, there were no signs of overt toxicity (mortality) observed within 48 hours of exposure (B. Wu et al., 2012). Although, the mean life span was significantly shortened in both sexes, with an apparent dose-response relationship to exposure of cerium (Huang et al., 2009). Higher concentrations of Cd and Hg resulted in larval mortality, with 100% mortality observed in some cases (Frat et al., 2021) and Hg(II) exposure resulted in a significant increase in fly mortality at the highest concentration after 24 hours, and at all concentrations - 30, 100, and 300 μM - after 48 hours of exposure (Paula et al., 2012). Ag NPs ingestion had dose-dependent effects on survivorship to the pupal stage. Uncoated particles were more toxic than coated ones, and smaller particles were more toxic than larger ones (Posgai et al., 2011). In contrast, TiO_2 NPs ingestion at doses up to 200 μg/mL and ZnO NPs did not significantly affected survivorship, time to pupation, or life span of flies (Anand et al., 2016; Posgai et al., 2011). Treatment with 1 mM of CeO_2 NPs did not significantly affect the survival of *D. melanogaster* exposed (Sarkar et al., 2021), and $CuCl_2$ was more lethal than CuO NPs (Budiyanti et al., 2022).

In terms of accumulation, few articles tested this parameter, but both male and female flies exposed to Cd (13–52 mg/L) had significantly higher Cd accumulation compared to the control group, accumulating 6.5–9.9 times more in females and 4.9–11.4 times more in males (Hu et al., 2019). Thus, there was a tendency for higher accumulation with $CuCl_2$ at lower concentrations (Budiyanti et al., 2022). This was also true in zebrafish, that even after 75 days of decontamination still had accumulated Cd (Arini et al., 2015).

Following the tendency, bisphenol C (BPC), bisphenol S (BPS), and BPZ had significant effects on the pupation rate, delaying the development from larvae to pupae by various days (Wang et al., 2023), that was also true for BPA (Sarkar et al., 2021). These results are comparable to the ones presented in Martínez et al. (2020), where the BPA exposure had both acute and chronic effects in the development of *D. rerio*. The long-term exposure to bisphenol also induced various abnormalities in the adult zebrafish behaviour (Ju Wang et al., 2015). Besides, the chronic exposure to BPA had serious negative effects in *Caenorhabditis elegans* (Zhou et al., 2016), including accelerated aging that followed the induction of oxidative stress in this nematode (Tan et al., 2015).

In Frat et al. (2023), all pollutants, except glyphosate, led to a lengthening of the larval stage duration, with the most pronounced delays observed at the highest pollutant concentrations and also in combinations, suggesting synergistic effects. Larvae and pupae exposed to chlordane exhibited significant developmental delays, leading to smaller body sizes and extended development times (Q. Wu et al., 2021). *Drosophila* exposed to ciprofloxacin showed significant reductions in pupation and eclosion (hatching) rates. In addition, larvae failed to develop normally, had significantly lower weights and smaller body sizes (Liu et al., 2019).

The proportion of larvae that reached eclosion and pupated were significantly reduced by atrazine exposure, and an accelerated adult emergence in both males and females affected adult body size. The allowed limit concentration of atrazine is 3 ppb and the highest concentration soluble in water is 20 ppm, with intermediate atrazine concentrations resulting in the fastest development time (Marcus & Fiumera, 2016).

A concentration of 10 mg/L of Ag, sufficient to kill bacteria, did not exhibit any acute toxic effects on any developmental stage of *Drosophila* and it did not prolong the development time. At a concentration of 20 mg/L of Ag, comparable to cytotoxic levels for human fibroblasts, acute toxic effects were observed, resulting in a decrease in the total number of hatched individuals. A concentration of 40 mg/L of Ag led to a prolonged development time, and concentrations of 60 to 100 mg/L significantly influenced the developmental stages of larvae and pupae, preventing the completion of the *Drosophila* development cycle. At 100 mg/L, 97% of larvae died, and no pupae were formed. Additionally, Ag NPs negatively affected the physical characteristics of adult flies, particularly their body size (Panacek et al., 2011).

Surprisingly, in Weiner et al. (2014) an increase in the growth rate of larvae was observed, most significantly in the lowest concentration treatment group (0.1 mg/L BPA), with the highest growth rate between 72 and 96 hours of development. BPA-induced growth abnormalities were consistent with disruptions in the insulin/insulin growth factor signalling (IIS) pathway, which plays a role in regulating larval size. Differences in exposure timing and method may account for these variations.

Cerium exposure was found to prolong the developmental time of *D. melanogaster*, especially at concentrations up to 26.3 µg/g (B. Wu et al., 2012). Correspondingly, high metal concentrations, particularly 20 mg/L of Cd, led to a significant increase in the time required to reach the pupal stage, suggesting a developmental delay. Unusual phenotypes, such as hatching adults with underdeveloped wings, were observed at 5 mg/L Hg exposure (Frat et al., 2021). Individuals exposed to copper during larval development had slower development times compared to the uncontaminated control group (Pölkki & Rantala, 2020) and a reduction in the number of eggs produced in both copper treatments (Budiyanti et al., 2022).

Activities of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) increased in ciprofloxacin-treated larvae, suggesting oxidative stress. This might be related to overproduction of reactive oxygen species (ROS) due to mitochondrial dysfunction (Liu et al., 2019). The same happened with BPA exposure, the effects were only alleviated by exposure to vitamin E (Chen et al., 2022). Similar to this, in Posgai et al. (2011) the levels of superoxide dismutase (SOD) and glutathione (GSH) were altered in flies fed with vitamin C besides the Ag NPs.

Exposure to Spectracide® led to increased protein carbonylation in both male and female flies, indicating oxidative stress (Chaudhuri et al., 2020). Exposure to Roundup® and POEA also led to an increase in carbonylated proteins, while glyphosate exposure did not, suggesting that is the herbicide formulation and surfactant that cause oxidative stress in flies (Bednářová et al., 2020). This opposed to Frat et al. (2023), where glyphosate exhibited neurotoxicity, endocrine disruption, and potential DNA damage, suggesting its potential classification as an endocrine-disrupting compound (EDC). These differences in studies' results could be related to changes in concentrations and variable methodologies. The study identified multiple molecular disturbances, including the modulation of heat shock proteins (HSPs), and oxidative stress, which were consistent with effects observed in vertebrates and mammals. It also proposed metallothionein genes (MTNs) as promising biomarkers, with high expression after exposure to mixtures containing heavy metals (Frat et al., 2023).

Chlorpyrifos exposure, at the highest concentration of 15 mg/L, caused DNA damage in *Drosophila* larvae, and a significant increase in ROS generation, which appeared to precede changes in apoptotic endpoints in the exposed organisms (Gupta et al., 2010). The same seems to happen in zebrafish exposed to chlorpyrifos and other pesticides, as we can see in Falfushynska et al. (2022). This suggests that both model organisms must be contemplated in additional studies on the oxidative stress mechanisms as tools for risk assessment.

Chlordane exposure resulted in abnormal glucose and lipid metabolism in 3rd instar larvae, suggesting a link between this exposure and insulin resistance in larvae. The study also observed increased oxidative stress in *Drosophila* larvae exposed to chlordane (Q. Wu et al., 2021).

In B. Wu et al. (2012), it was detected an increase in the expression of Hsp70 (a stress-inducible heat shock protein) in a concentration-dependent manner with Cerium exposure. Exposure to 0.45 and 1.65 µg/g of cerium stimulated antioxidant enzyme activity (SOD and CAT) and concentrations of 26.3, 104, and 429 µg/g inhibited SOD and CAT activity levels, indicating that cerium induced oxidative damage (Huang et al., 2009). Cerium exposure also led to an up-regulation of p38 and p53 expression, two conserved signalling proteins involved in various cellular pathways, including those related to DNA damage and apoptosis (B. Wu et al., 2012).

BPA exposure induced apoptosis in *Drosophila* gut tissues, decreased acetylcholinesterase levels, which is important for neurological function, and increased the lipid peroxides – a marker of oxidative stress. Co-treatment with CeO_2 NPs improved these effects, suggesting a role in mitigating oxidative stress and preventing cell death (Sarkar et al., 2021). In a similar study by Oliveira Pereira et al. (2021), *Daphnia magna* was exposed to sub-lethal concentrations of BPA, BPF, and BPS, resulting in changes on metabolic pathways that caused protein synthesis impairment. These and the previous results regarding bisphenol exposure demonstrate its highly negative impacts in organisms of both aquatic and terrestrial environments.

This oxidative stress is a common response to heavy metal exposure. Cd had a more pronounced effect on gene expression compared to Hg, and the co-exposure showed consistently synergistic effects on gene expression, especially in the regulation of oxidative stress mechanisms (Frat et al., 2021). This was also proved by Paula et al. (2012), where the expression levels of metal response genes (SOD, CAT) were not significantly different between mercury treated and control flies, and by Hu et al. (2019) where cadmium exposure resulted in significantly increased acetylcholinesterase (AChE) activity in both male and female flies. Nonetheless, exposure to Cd and Hg led to a significant increase in the activities of SOD, CAT, and in the ROS levels in the *D. melanogaster*'s Malpighian tubules in a concentration-dependent manner (Saini et al., 2020). The chronic toxicity of ZnO NPs had mutagenic effects, leading to genotoxicity and the presence of abnormal phenotypes in the offspring that increased with the concentration (Anand et al., 2016).

Like in the exposures evaluating survival rates of *D. melanogaster* to Roundup® and POEA, in Bednářová et al. (2020) the same compounds significantly reduced the reproductive fitness (fecundity) of *D. melanogaster*, while pure glyphosate did not have a similar effect. One possible explanation is that Roundup® and POEA induce oxidative stress, leading to proapoptotic events in reproductive tissues.

The results suggest that ecologically relevant doses of larval atrazine exposure affected male reproductive performance in *D. melanogaster*, essentially in intermediate concentrations. Differences in the number of adults that emerged were attributed to variations in the number of eggs laid (Vogel et al., 2014). However, the effect of atrazine exposure on female mating and remating rates was not consistent, because females exposed to the highest concentration of atrazine laid more eggs and produced more offspring (Marcus & Fiumera, 2016).

A dose-dependent decrease in fertility was observed in both female and male *Drosophila melanogaster*, noticeable from 16 to 1024 mg/L of cerium, suggesting a potential toxic oxidative mechanism (Huang et al., 2009). Cd exposure led to increased mating latency on female flies, reducing the number of eggs laid after mating. In contrast, male fecundity was not significantly affected by Cd exposure (Hu et al., 2019). In other hand, Ag NPs ingestion during the larval stage reduced adult mating success, even at lower doses (Posgai et al., 2011).

The chronic toxicity assay, at a lower concentration of 5 mg/L of Ag extended over 8 consecutive generations. The first generation was not significantly impacted, but from F2 to F4 there was a decrease in hatched individuals. Starting from the F5 generation, the total number of hatched adults gradually increased, reaching the same level as the control in the F7 generation. The study suggested that *Drosophila* exposed to low concentrations of Ag NPs adapted over generations, reversing the negative effects in fecundity (Panacek et al., 2011). Equivalently, larval exposure to copper led to reduced fecundity in adult flies with additional trans-generational effects on their offspring. But when offspring was under copper-contaminated conditions for two generations they showed improved fecundity.

Trans-generational effects on fecundity could be a result of short-term acclimatization to heavy metal exposure through phenotypic plasticity (Pölkki & Rantala, 2020). However, the ultimate mechanisms and long-term consequences of these trans-generational effects remain unclear and require further investigation.

Cd stress induced apoptosis in the wing disc of 3rd instar larvae in the first generations (F0-F2) but not in F3 and F4 flies, indicating that Cd's inhibitory effects on gene expression could be transmitted to offspring for 2-3 generations after Cd removal (Sun et al., 2022). Wing morphology analysis showed significant differences among females from different generations treated with TiO_2 NPs. Generational differences were observed, with the most prominent differences occurring in the later generations both in male and female flies (Cvetković et al., 2020). In Anand et al. (2016), the chronic effects of ZnO NPs affected the progeny flies exhibiting abnormal phenotypes, with most showing wing deformation, but also deformed thorax, and single wing.

Exposure to BPA and its derivates induced gender-specific behaviour defects, with BPC having the most substantial influence. Female fruit flies exhibited abnormal social behaviour at higher BPA concentrations, while both male and female fruit flies showed social interaction deficits with BPC exposure (Wang et al., 2023). Larvae and adult flies exposed to BPA exhibited a decline in locomotion, and the most significant decrease was observed at higher BPA concentrations (Chen et al., 2022; Sarkar et al., 2021), but the co-treatment with CeO_2 NPs prevented these deficits (Sarkar et al., 2021).

Vertical climbing (negative geotaxis) and general fly movement were decreased in both male and female flies exposed to Spectracide®. Herbicide-exposed flies took more time to climb a 5 cm distance against gravity and the number of flies crossing the 5 cm mark was reduced (Chaudhuri et al., 2020). Correspondingly, flies exposed to 100 µM Hg(II) for 48 hours took longer to travel 8 cm in vials, while flies treated with 300 µM $HgCl_2$ were unable to climb 8 cm within the observation period (Paula et al., 2012). Exposure to 1 mM ZnO NPs significantly impaired climbing behaviour in flies compared to control, but no significant effect was observed with lower (0.1 mM) or higher (10 mM) concentrations (Anand et al., 2016), the same happened to both copper compounds exposure (Budiyanti et al., 2022).

Although the adverse effects, essentially regarding the higher concentrations of chemicals, were expected there are some interesting discoveries that are important to mention, including the vitamin C capacity to reverse some of the toxic effects of Ag NPs (Posgai et al., 2011), the potential benefits of CeO_2 NPs as well as the antioxidant vitamin E in mitigating the detrimental effects of BPA exposure (Chen et al., 2022; Sarkar et al., 2021), and the potential health impacts of herbicides formulations beyond the active ingredient glyphosate (Bednářová et al., 2020). Further studies are recommended to explore the therapeutic and ecological potential of this in more detail.

5. Conclusions

This investigation raises awareness about the overuse and misuse of pesticides, bisphenols, and metals in environmental settings, due to potential long-term impacts that include disruption of genetic material and adverse effects on development, reproduction, and behaviour. This research sheds light on the importance of studying non-vertebrate organisms as potential targets for chemical contamination.

Essentially, this study proved the importance of *Drosophila melanogaster* as a model organism for ecological and health risk assessment. For example, developmental delays occurring at low concentrations, indicates the sensibility of the *Drosophila* model in detecting contamination at these levels. Additionally, fecundity, development, gene expression, and defence responses were identified as valuable tools for assessing chemical toxicity and resistance in organisms. The study also emphasized the value of integrative protocols that combine molecular analyses and biological phenotypes to comprehensively assess pollutant toxicity.

Nevertheless, it is important to highlight the limitations found. This review underlines the importance of considering more chronic toxicity testing in *Drosophila melanogaster*, and combined exposures when assessing the impact of pollutants on organisms. The research also highlights the importance of studying toxicity at multiple levels of biological organization, combining molecular biomarkers, genetic factors, biological responses, and potential variations in susceptibility among different *Drosophila* genotypes. This integrated approach could have implications for setting new standards for whole-organism testing and furthering our understanding of the effects of contaminants.

In summary, this systematic review of studies involving the exposure of *D. melanogaster* to a diverse range of substances, that include heavy metals, pesticides, nanoparticles, and antibiotics, revealed significant insights into the potential ecological, health, and developmental risks associated with these agents. These findings emphasize the value of *Drosophila* as a model organism for ecotoxicological evaluation, offering valuable perspectives on adverse effects at multiple levels of biological organization. Moreover, they underscore the importance of considering long-term and combined exposures in assessing the impact of pollutants on organisms and the need for continued research into the molecular mechanisms underlying these effects. This body of work informs our understanding of the intricate interactions between organisms and environmental contaminants, highlighting the significance of adopting holistic approaches in risk assessment and the development of safety guidelines.

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Chapter III: Final Considerations and Future Perspectives

The climate change and ongoing environmental degradation have raised disturbing questions about environmental protection and the preservation of animal and human welfare. In this case, the use of ecotoxicological tests provides a solid scientific basis for developing strategies to mitigate the effects of environmental contamination.

In ecotoxicology, *Drosophila melanogaster* has proven to be an incredibly practical, versatile, and economical model organism due to its rapid development, ease of maintenance and very well-known genetics. This makes the fruit fly an essential tool for assessing the impact of contaminants on all levels of biological organisation.

In this dissertation, the selected studies have highlighted the adverse effects of several pollutants, including pesticides, metals, metal nanoparticles, bisphenols, and pharmaceuticals, on *Drosophila*. Despite these central findings, they also showed some research deficiencies, namely in transposing these discoveries to a real ecological context that includes exposure to different contaminants and at the same time constant interaction with other species.

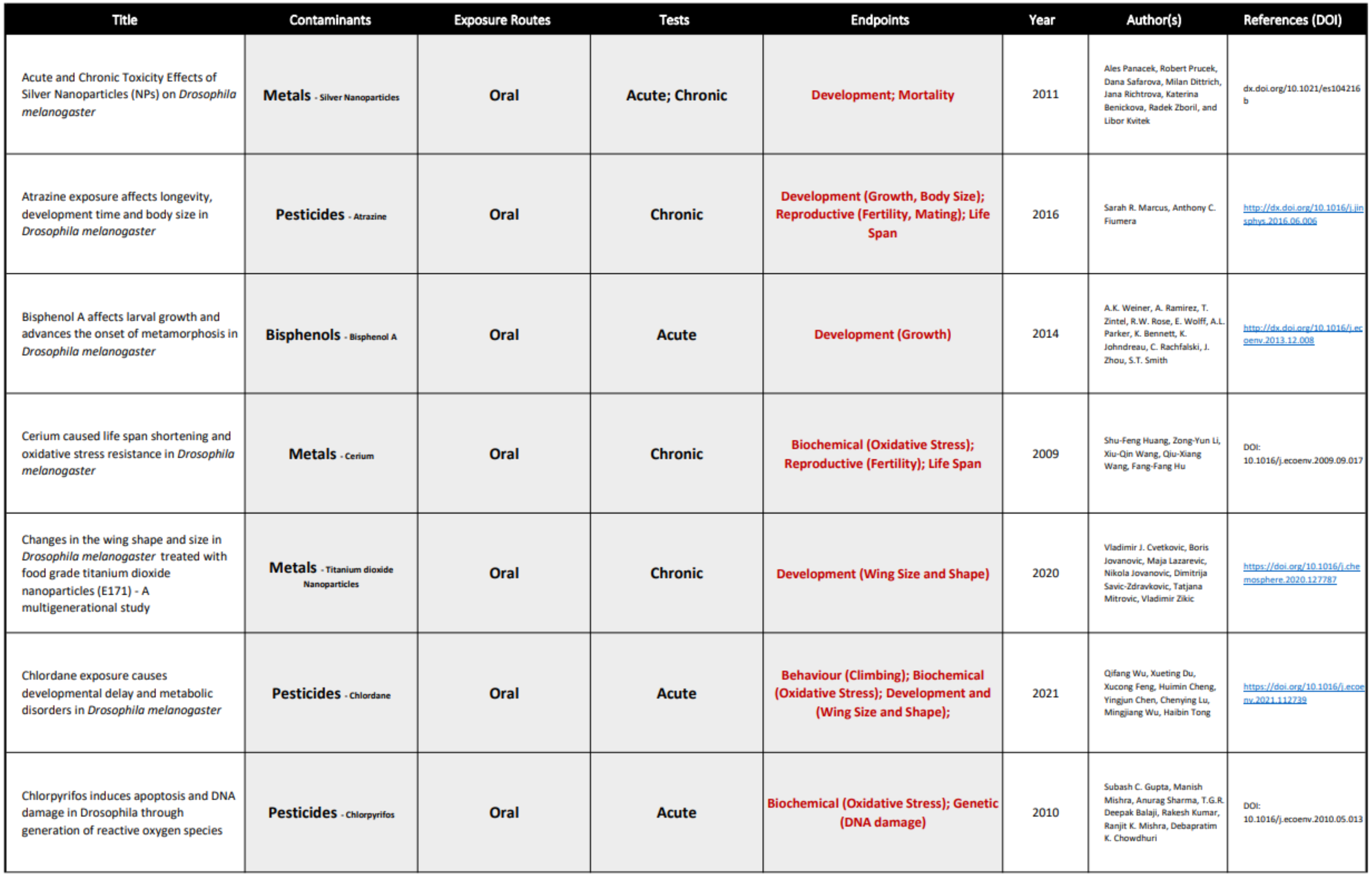
For this reason, it is important to conduct additional studies that highlight the importance of fruit fly responses to these substances to help clarify the intricacies of toxicity and how these results can be transposed to humans and other organisms. Carrying out more long-term studies with *Drosophila* can provide new insights into the chronic effects of contaminants as well as the potential adaptation of flies to these conditions. In addition, it seems essential to expand scientific research to include studies of interactions between species and the dynamics of food chain, which intends to improve our knowledge in what way pollutants can affect entire ecosystems. These trials should involve fieldwork on community-level relationships, that can provide valuable insights into the wide-ranging ecological implications of these chemicals. Given that organisms are simultaneously exposed to various contaminants in the environment, there is also a need to carry out more studies on the potential interactions between these substances and their dose-response relations. Finally, understanding the molecular and genetic mechanisms that are specifically linked to the observed effects can not only improve our knowledge of the issue of environmental contamination, but also provide support for prevention and intervention measures.

As we know, ecotoxicology is constantly evolving, whether due to the appearance of new substances in the environment or the discovery of innovative ways of analysing the reactions to environmental stress factors. Therefore, with the growing technological and scientific advances in Genetics and Molecular Biology, the integration of a model organism such as *D. melanogaster* into broader collaborations with geneticists, ecologists and environmentalists is pondered as a promising opportunity for future research.

Hence, we can conclude that the use of *D. melanogaster* in ecotoxicological tests has established itself as a powerful approach to better understand the ecological and genetic outcomes of exposure to contaminants. *Drosophila* ecotoxicology therefore holds promise to address the complex environmental challenges caused by pollutants and to develop our understanding of the impacts on ecosystems and human health itself, consequently transforming the environmental risk assessments.

Supplementary Material

Table S1 - List of 27 scientific papers used in the systematic review of Chapter II, chosen based on the inclusion criteria and divided and categorised according to: i) contaminants used; ii) exposure routes; iii) type of tests performed; iv) endpoints measured.



Title	Contaminants	Exposure Routes	Tests	Endpoints	Year	Author(s)	References (DOI)
Acute and Chronic Toxicity Effects of Silver Nanoparticles (NPs) on *Drosophila melanogaster*	**Metals** - Silver Nanoparticles	Oral	Acute; Chronic	Development; Mortality	2011	Ales Panacek, Robert Prucek, Dana Safarova, Milan Dittrich, Jana Richtrova, Katerina Benickova, Radek Zboril, and Libor Kvitek	dx.doi.org/10.1021/es104216b
Atrazine exposure affects longevity, development time and body size in *Drosophila melanogaster*	**Pesticides** - Atrazine	Oral	Chronic	Development (Growth, Body Size); Reproductive (Fertility, Mating); Life Span	2016	Sarah R. Marcus, Anthony C. Fiumera	http://dx.doi.org/10.1016/j.jinsphys.2016.06.006
Bisphenol A affects larval growth and advances the onset of metamorphosis in *Drosophila melanogaster*	**Bisphenols** - Bisphenol A	Oral	Acute	Development (Growth)	2014	A.K. Weiner, A. Ramirez, T. Zintel, R.W. Rose, E. Wolff, A.L. Parker, K. Bennett, K. Johndreau, C. Rachfalski, J. Zhou, S.T. Smith	http://dx.doi.org/10.1016/j.ecoenv.2013.12.008
Cerium caused life span shortening and oxidative stress resistance in *Drosophila melanogaster*	**Metals** - Cerium	Oral	Chronic	Biochemical (Oxidative Stress); Reproductive (Fertility); Life Span	2009	Shu-Feng Huang, Zong-Yun Li, Xiu-Qin Wang, Qiu-Xiang Wang, Fang-Fang Hu	DOI: 10.1016/j.ecoenv.2009.09.017
Changes in the wing shape and size in *Drosophila melanogaster* treated with food grade titanium dioxide nanoparticles (E171) - A multigenerational study	**Metals** - Titanium dioxide Nanoparticles	Oral	Chronic	Development (Wing Size and Shape)	2020	Vladimir J. Cvetkovic, Boris Jovanovic, Maja Lazarevic, Nikola Jovanovic, Dimitrija Savic-Zdravkovic, Tatjana Mitrovic, Vladimir Zikic	https://doi.org/10.1016/j.chemosphere.2020.127787
Chlordane exposure causes developmental delay and metabolic disorders in *Drosophila melanogaster*	**Pesticides** - Chlordane	Oral	Acute	Behaviour (Climbing); Biochemical (Oxidative Stress); Development and (Wing Size and Shape);	2021	Qifang Wu, Xueting Du, Xucong Feng, Huimin Cheng, Yingjun Chen, Chenying Lu, Mingjiang Wu, Haibin Tong	https://doi.org/10.1016/j.ecoenv.2021.112739
Chlorpyrifos induces apoptosis and DNA damage in Drosophila through generation of reactive oxygen species	**Pesticides** - Chlorpyrifos	Oral	Acute	Biochemical (Oxidative Stress); Genetic (DNA damage)	2010	Subash C. Gupta, Manish Mishra, Anurag Sharma, T.G.R. Deepak Balaji, Rakesh Kumar, Ranjit K. Mishra, Debapratim K. Chowdhuri	DOI: 10.1016/j.ecoenv.2010.05.013

Chronic exposure of zinc oxide nanoparticles causes deviant phenotype in *Drosophila melanogaster*	**Metals** - Zinc oxide Nanoparticles	**Oral**	**Chronic**	**Behaviour (Climbing); Biochemical (Oxidative Stress); Genetic (DNA damage); Life Span**	2016	Avnika Singh Anand, Dipti N. Prasad, Shashi Bala Singh, Ekta Kohli	http://dx.doi.org/10.1016/j.jhazmat.2016.12.040
Development of a *Drosophila melanogaster* based model for the assessment of cadmium and mercury mediated renal tubular toxicity	**Metals** - Cadmium and Mercury	**Oral**	**Acute**	**Biochemical (Oxidative Stress)**	2020	Sanjay Saini, Lavi Rani, Neha Shuklaa, Monisha Banerjee, Debapratim Kar Chowdhuri, Naveen Kumar Gautam	https://doi.org/10.1016/j.ecoenv.2020.110811
Developmental neurotoxic effects of bisphenol A and its derivatives in *Drosophila melanogaster*	**Bisphenols** - Bisphenols	**Oral**	**Acute**	**Behaviour (Climbing); Development; Mortality**	2023	Jie Wang, Chunyan Wu, Xing Zhang, Yuanyuan Song, Binquan Wang, Ke Zhang, Mingkuan Sun	https://doi.org/10.1016/j.ecoenv.2023.115098
Differential toxicity of silver and titanium dioxide nanoparticles on *Drosophila melanogaster* development, reproductive effort, and viability: Size, coatings and antioxidants matter	**Metals** - Titanium dioxide and Silver Nanoparticles	**Oral**	**Acute**	**Biochemical; Development; Reproductive (Fertility, Mating); Mortality**	2011	Ryan Posgai, Caitlin B. Cipolla-McCulloch, Kyle R. Murphy, Saber M. Hussain, John J. Rowe, Mark G. Nielsen	http://dx.doi.org/10.1016/j.chemosphere.2011.06.040
Effects of atrazine exposure on male reproductive performance in *Drosophila melanogaster*	**Pesticides** - Atrazine	**Oral**	**Acute**	**Development; Reproductive (Fertility, Mating)**	2014	Andrea Vogel, Harper Jocque, Laura K. Sirot, Anthony C. Fiumera	http://dx.doi.org/10.1016/j.jinsphys.2014.11.002
Effects of cadmium on fecundity and defence ability of *Drosophila melanogaster*	**Metals** - Cadmium	**Oral**	**Acute**	**Biochemical (Enzyme Activity); Genetic; Reproductive (Fertility, Fecundity)**	2019	Xiaoyu Hu, Weili Fu, Xingran Yang, Yun Mu, Wei Gu, Min Zhang	https://doi.org/10.1016/j.ecoenv.2019.01.029
Effects of Hg(II) Exposure on MAPK Phosphorylation and Antioxidant System in *D. melanogaster*	**Metals** - Mercury	**Oral**	**Acute**	**Behaviour (Movement); Biochemical (Enzyme Activity); Mortality**	2012	M. T. Paula, A. P. Zemolin, A. P. Vargas, R. M. Golombieski, E. L. S. Loreto, A. P. Saidelles, R. S. Picoloto, E. M. M. Flores, A. B. Pereira, J. B. T. Rocha, T. J. S. Merritt, J. L. Franco,2 T. Posser	DOI: 10.1002/tox.21788

Establishing a system with *Drosophila melanogaster* (Diptera: Drosophilidae) to assess the non-target effects of gut-active insecticidal compounds	**Pesticides** - Cryolite	**Oral**	**Acute**	**Development and (Wing Size); Survival**	2016	Simone Haller, Michael Meissle, Jörg Romeis	DOI: 10.1007/s10646-016-1722-y
Exposure to bisphenol A induced oxidative stress, cell death and impaired epithelial homeostasis in the adult *Drosophila melanogaster* midgut	**Bisphenols** - Bisphenol A	**Oral**	**Chronic**	**Behaviour (Climbing); Biochemical (Enzyme Activity)**	2022	Zhi Chen, Fen Wang, Di Wen, Ren Mu	https://doi.org/10.1016/j.ecoenv.2022.114285
Exposure to copper during larval development has intra- and trans-generational influence on fitness in later life	**Metals** - Copper	**Oral**	**Acute; Chronic**	**Development; Reproductive (Fecundity)**	2020	Mari Polkki, Markus J. Rantala	https://doi.org/10.1016/j.ecoenv.2020.111133
Exposure to Spectracide® causes behavioral deficits in *Drosophila melanogaster* : Insights from locomotor analysis and molecular modeling	**Pesticides** - Spectracide and Atrazine	**Oral**	**Acute**	**Behaviour (Movement); Biochemical (Oxidative Stress)**	2020	Ankur Chaudhuri, Roishinique Johnson, Kuntol Rakshit, Andrea Bednarova, Kimberly Lackey, Sibani Sen Chakraborty, Natraj Krishnan, Anathbandhu Chaudhuri	https://doi.org/10.1016/j.chemosphere.2020.126037
Impact of single and combined exposure to priority pollutants on gene expression and post-embryonic development in *Drosophila melanogaster*	**Bisphenols** - Bisphenol A; **Pesticides** - Glyphosate; **Metals** - Mercury and Cadmium	**Oral; Contact**	**Acute**	**Biochemical (Oxidative Stress); Development; Survival**	2023	La¨etitia Frat, Thomas Chertemps, Elise Pesce, Françoise Bozzolan, Matthieu Dacher, Rosario Planello, Oscar Herrero, Lola Llorente, Didier Moers, David Siaussat	https://doi.org/10.1016/j.ecoenv.2022.114491
Influence of copper treatment on bioaccumulation, survival, behavior, and fecundity in the fruit fly *Drosophila melanogaster* : Toxicity of copper oxide nanoparticles differ from dissolved copper	**Metals** - Copper and Copper Oxide Nanoparticles	**Oral**	**Acute; Chronic**	**Behaviour (Climbing); Development; Reproductive; Mortality**	2022	Dwi Sari Budiyanti, Morten Erik Moeller, Amalie Thit	https://doi.org/10.1016/j.etap.2022.103852
Role of cerium oxide nanoparticles in improving oxidative stress and developmental delays in *Drosophila melanogaster* as an in-vivo model for bisphenol a toxicity	**Bisphenols** - Bisphenol A; **Metals** - Cerium oxide Nanoparticles	**Oral**	**Acute; Chronic**	**Behaviour (Crawling); Biochemical; Development; Life Span**	2021	Arkajyoti Sarkar, Tharun Selvam Mahendran, Aasha Meenakshisundaram, Rushenka Vashti Christopher, Pallavi Dan, Vignesh Sundararajan, Nishant Jana, Devanand Venkatasubbu, Sahabudeen Sheik Mohideen	https://doi.org/10.1016/j.chemosphere.2021.131363

Single and mixed exposure to cadmium and mercury in *Drosophila melanogaster* : Molecular responses and impact on post-embryonic development	**Metals** - Cadmium and Mercury	**Oral; Contact**	**Acute; Chronic**	**Development; Genetic**	2021	Laetitia Frat, Thomas Chertemps, Elise Pesce, Françoise Bozzolan, Matthieu Dacher, Rosario Planello, Oscar Herrero, Lola Llorente, Didier Moers, David Siaussat	https://doi.org/10.1016/j.ecoenv.2021.112377
The Joint Toxicity of Type I, II, and Nonester Pyrethroid Insecticides	**Pesticides** - Permethrin, Etofenprox and Cypermethrin	**Contact**	**Acute**	**Mortality**	2012	Jerome J. Schleier and Robert K. D. Peterson	DOI: http://dx.doi.org/10.1603/EC11267
The potential toxic effects of cerium on organism: cerium prolonged the developmental time and induced the expression of Hsp70 and apoptosis in *Drosophila melanogaster*	**Metals** - Cerium	**Oral**	**Acute**	**Development; Genetic**	2012	Bin Wu, Di Zhang, Dan Wang, Chunyan Qi, Zongyun Li	DOI: 10.1007/s10646-012-0960-x
The surfactant polyethoxylated tallowamine (POEA) reduces lifespan and inhibits fecundity in *Drosophila melanogaster* - In vivo and in vitro study	**Pesticides** - Glyphosate; Roundup and Polyethoxylated tallow amine	**Oral; Contact**	**Acute; Chronic**	**Biochemical (Oxidative Stress); Reproductive (Fecundity); Life Span**	2020	Andrea Bednářová, Maximillian Kropfa, Natraj Krishnan	https://doi.org/10.1016/j.ecoenv.2019.109883
Toxicological effects of ciprofloxacin exposure to *Drosophila melanogaster*	**Pharmaceuticals** - Ciprofloxacin	**Oral**	**Acute**	**Biochemical (Oxidative Stress); Development; Mortality; Life Span**	2019	Jinyue Liu, Xiaoqin Li, Xing Wang	https://doi.org/10.1016/j.chemosphere.2019.124542
Transgenerational inheritance of wing development defects in *Drosophila melanogaster* induced by cadmium	**Metals** - Cadmium	**Oral**	**Acute**	**Development (Wing Size and Shape); Genetic**	2022	Liran Sun, Yun Mu, Lu Xu, Xiaobing Han, Wei Gu, Min Zhang	https://doi.org/10.1016/j.ecoenv.2022.114486