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Drosophila: A Promising Model for Evaluating the Toxicity of Environmental Pollutants

Drosophila: Çevresel Kirleticilerin Toksisitesini Değerlendirmek İçin Umut Veren Bir Model

Eşref Demir*, Fatma Turna Demir

Department of Medical Services and Techniques, Medical Laboratory Techniques Programme, Vocational School of Health Services, Antalya Bilim University, 07190, Dosemealti, Antalya, Turkey

Abstract

Environmental contamination has now become a major global issue with adverse effects on our health and food security. Humans and animals are being exposed to debilitating levels of contamination on a daily basis. Across the globe, air pollution alone causes millions of premature deaths annually, mainly from lung cancer, chronic obstructive pulmonary disease (COPD), stroke, heart failure, and respiratory infections, and according to World Health Organization (WHO), 99% of humanity breathes air containing contaminants above recommended levels. The United Nations (UN) has identified "a pollution-free planet" goal among its three pillars, besides climate change and biodiversity for 2022–2025. In order to mitigate contamination and relieve our burden of pollution-related disease, we need to devise target-specific strategies. To that end, risk assessment of each chemical and natural contaminants and solid evidence from toxicity studies appear to be of paramount importance. Meticulous efforts should be made to look into possible mechanisms of action for each pollutant and detect their toxic potential and safe limits through comprehensive *in vivo* animal testing. Various factors such as high operational costs and ethical issues concerning the use of higher vertebrates frustratingly restrict the efficient use of traditional *in vivo* testing. Therefore, simpler and more dynamic model organisms like Drosophila melanogaster could be favored for toxicity studies, as 75% of the genes related with human diseases are known to have homologs in *D. melanogaster,* which facilitates research into different anomalies. This review aims to present the picture of studies regarding environmental pollutants that employed using *D. melanogaster,* attempting to offer a comprehensive analysis of risks involved in exposure to environmental pollutants.

Keywords: *Drosophila melanogaster,* Environmental pollutants, Risk assessment, Ecotoxicity, *In vivo* model organism, Health

Öz

Çevre kirliliği artık sağlığımız ve gıda güvenliğimiz üzerinde olumsuz etkileri olan önemli bir küresel sorun haline gelmiştir. İnsanlar ve hayvanlar, günlük olarak kendilerini zayıflatıcı kontaminasyon seviyelerine maruz kalmaktadır. Dünya genelinde tek başına hava kirliliği başlıca akciğer kanseri, kronik obstrüktif akciğer hastalığı (KOAH), felç, kalp yetmezliği ve solunum yolu enfeksiyonları sebebiyle her yıl milyonlarca erken ölüme neden olmakta ve Dünya Sağlık Örgütü (WHO)'ne göre, insanlığın %99'u önerilen seviyelerin üzerinde kirletici içeren havayı solumaktadır. 2022-2025 için iklim değişikliği ve biyolojik çeşitliliğin yanı sıra Birleşmiş Milletler (BM), üçüncü desteğini "kirlilik içermeyen bir gezegen" hedefi olarak belirlemiştir. Kirliliği azaltmak ve kirlilikle ilgili hastalık yükümüzü hafifletmek için hedefe özel stratejiler geliştirmemiz gerekmektedir. Bu amaçla, her bir kimyasal ve doğal kirleticinin risk değerlendirmesi ve toksisite çalışmalarından elde edilen somut kanıtlar çok önemli görünmektedir. Her kirletici için olası etki mekanizmalarını araştırmak ve kapsamlı *in vivo* hayvan testleri yoluyla bu kirleticilerin toksik potansiyellerini ve güvenli sınırlarını saptanmak için titiz çaba gösterilmelidir. Yüksek çalışma maliyetleri ve daha yüksek omurgalıların kullanımına ilişkin etik sorunlar gibi çeşitli faktörler, geleneksel *in vivo* testlerin verimli kullanımını engelleyici bir şekilde kısıtlamaktadır. Bu nedenle, toksisite çalışmaları için *D. melanogaster* gibi daha basit ve dinamik model organizmalar tercih edilebilir, çünkü insan hastalıklarıyla ilgili genlerin %75'inin *D. melanogaster*'de homologları olduğu bilinmektedir, bu da farklı anomalilerin araştırılmasını kolaylaştırmaktadır. Bu derleme, D. melanogaster kullanılarak çevresel kirleticilerle ilgili çalışmaları belirterek çevresel kirleticilere maruz kalmayla ilgili risklerin kapsamlı bir analizini sunmayı amaçlamaktadır.

Anahtar Kelimeler: *Drosophila melanogaster,* Çevresel kirleticiler, Risk değerlendirmesi, Ekotoksisite, *In vivo* model organizma, Sağlık

*Corresponding author: esref.demir@antalya.edu.tr

Eşref Demir orcid.org/0000-0002-2146-7385
Fatma Turna Demir orcid.org/0000-0001-8045-8641

1. Introduction

The chemical sector is one of the largest industries, and global plastics production, which totaled 368 million metric tons in 2019, is expected to grow by 4.4% in 2021 according to Baden Aniline and Soda Factory (BASF) Online Report 2020 (https://report.basf.com/2020/en/). Many consumer products, including foods, clothes, cleaning products, paints, cosmetics, and electronics contain chemical contaminants, and living organisms are often exposed to these contaminants through food intake and dermal contact, as well as inhalation and transfer via placenta. Toxicity from exposure to a range of environmental contaminants has also become a major health concern throughout the world. Epidemiological research has revealed that such exposure during prenatal period and early life deteriorates the healthy development of fetus or embryo (conceptus) as well as newborns (Wigle et al. 2008, Kadawathagedara et al. 2018). The common complications resulting from such exposure include embryonic mortality, fetal loss, intrauterine growth restriction, preterm birth, birth defects, childhood diseases, neuropsychological deficits, premature or delayed sexual maturation and certain adult cancers (Wigle et al. 2008).

Humans and animals could be exposed to a mixture of environmental contaminants that may act on several organ systems through different mechanisms. For instance, previous research has shown that endocrine disruptors working through distinct toxicity mechanisms to impair hormone-dependent signaling pathways in differentiating tissues produce cumulative dose-additive effects independent of the mechanism (Rider et al. 2012, Karwacka et al. 2019). Multicomponent mixtures of endocrine-disturbing chemicals have been found to act as antagonists which could disrupt pathways of oestrogen, androgen, and other hormones (Kortenkamp et al. 2007). Another important mechanism of action for environmental chemicals appears to be oxidative stress induced by reactive oxygen species (ROS) (Wells et al. 2009, Luo et al. 2010).

Susceptibility of embryo or fetus to developmental disturbances related to environmental contaminants depends on exposition time and dose. Besides, compromised antioxidant defense leaves the embryo prone to oxidative damage through *in utero* exposure to such contaminants, because the embryo lacks fully developed antioxidant enzymes and detoxification pathways (Al-Gubory and Garrel 2012). For this reason, the relationship of environmental contaminants and impaired fetal growth attracts significant attention from the fertility and reproductive health sector.

Several types of environmental contaminants may combine to produce different chemical mixtures, which include pesticides, nanopesticides, nanoparticles, micro-nanoplastics, heavy metals, pharmaceutical products, xenoestrogens, and various others (indoor air pollutants, haloalkanes, hydrazines, smog, etc.). Here we present examples of natural contaminants and synthetic compounds that might pose significant risks to human health, as they have a common mechanism of toxicity, with the idea that once we have deciphered this mechanism, we can make huge strides towards prevention and mitigation of this global problem.

2. Aims and Scope

Today, disordered urban growth constantly produces industrial, domestic, and agricultural waste that may contain harmful mixtures of chemicals, which tend to persist in nature, polluting our air, soil and freshwater resources. Therefore, research into possible mechanisms of action for environmental contaminants is of vital importance for the well-being of humans and animals (Bianchi et al. 2015). Comprehensive investigation of chemical contaminants for possible injurious effects through *in vivo* animal testing seems to be critical. Several factors such as high operational costs and ethical issues tend to restrict the use of traditional *in vivo* testing, therefore simpler experimental models like roundworms, zebrafish, and fruit flies are favoured, and however *Drosophila melanogaster* (fruit fly) stands out as an ideal model for cytotoxicity and genotoxicity studies. As we share a significant amount of genes with fruit flies - more than 60% of their DNA is identical to that of humans, almost 75% of the genes associated with human diseases have functional homologs in *D. melanogaster* (Demir 2020a, 2021b). Another advantage of *Drosophila* is that it shares various basic biological and physiological mechanisms and molecular pathways with mammals (Demir 2021b), which makes this insect an excellent model organism for various fields, including genotoxicity studies (Demir 2021b), and neurotoxicity screening (Rand 2010). *D. melanogaster* also offers a series of advantages over rodents, including rapid life cycle, ease of culturing, low costs, high fecundity, and simple genetics with only four pairs of chromosomes. Most importantly, ethical issues associated with vertebrate animals do not apply to fruit flies.

Another important point is that the microbiota plays a critical role in certain aspects of *Drosophila* physiology such as growth and reproduction (Charroux and Royet 2012).

Drosophila also features a specific microbiota with key roles in its physiology and pathology (Kuraishi et al. 2013) and changes in the gut microbiota can affect the health status of *Drosophila*, with changes in both growth and body size (Shin et al. 2011). This opens a new field to study harmful effects of environmental contaminants like micro-nano particles, as shown by research reporting that exposure to nanoparticles caused a drastic reduction in the diversity of the gut microbiota in *Drosophila* (Han et al. 2014).

Chronic exposure to environmental contaminants is known to be associated with several health conditions, including cancer, impaired immune and reproductive function, as well as imbalanced gastrointestinal microbiota, which regulates a range of host metabolic and immune processes. Here we discuss possible effects of various contaminants abundant in the environment and their impact on different ecosystems, addressing the bidirectional relationship between *D. melanogaster* and environmental contaminants to investigate their toxicological relevance in the case of *Drosophila* as an *in vivo* model. The substantial increase in the amount of contaminants naturally intensifies our exposure to such contaminants, raising concerns over their possible adverse effects on humans and non-target organisms. This makes the risk assessment for their toxicity and genotoxicity imperative, as we urgently require conclusive evidence on their safety or toxicity/genotoxicity (Figure 1). Table 1 indicates that widespread risk assessment methods for mixtures of environmental pollutants and mixtures whose effects have been investigated in literature. The aims of this review are to present a comprehensive overview of different studies carried out with *D. melanogaster* and the potential risk of environmental pollutants exposure to health, and to demonstrate the advantages of using *Drosophila*.

3. Common Pollutants

3.1. Pesticides and Nanopesticides

Previous toxicity experiments have revealed that exposure to pesticides could result in lipid peroxidation and DNA damage in humans (Kapeleka et al. 2021) and in rodents (Milić et al. 2018). Experiments on aquatic animals show that oxidative stress might play a serious role in the mechanism by which pesticides create risks for developmental toxicity (Kumar et al. 2021).

The widespread use of pesticides and nanopesticides has been raising concerns over the last decades, since

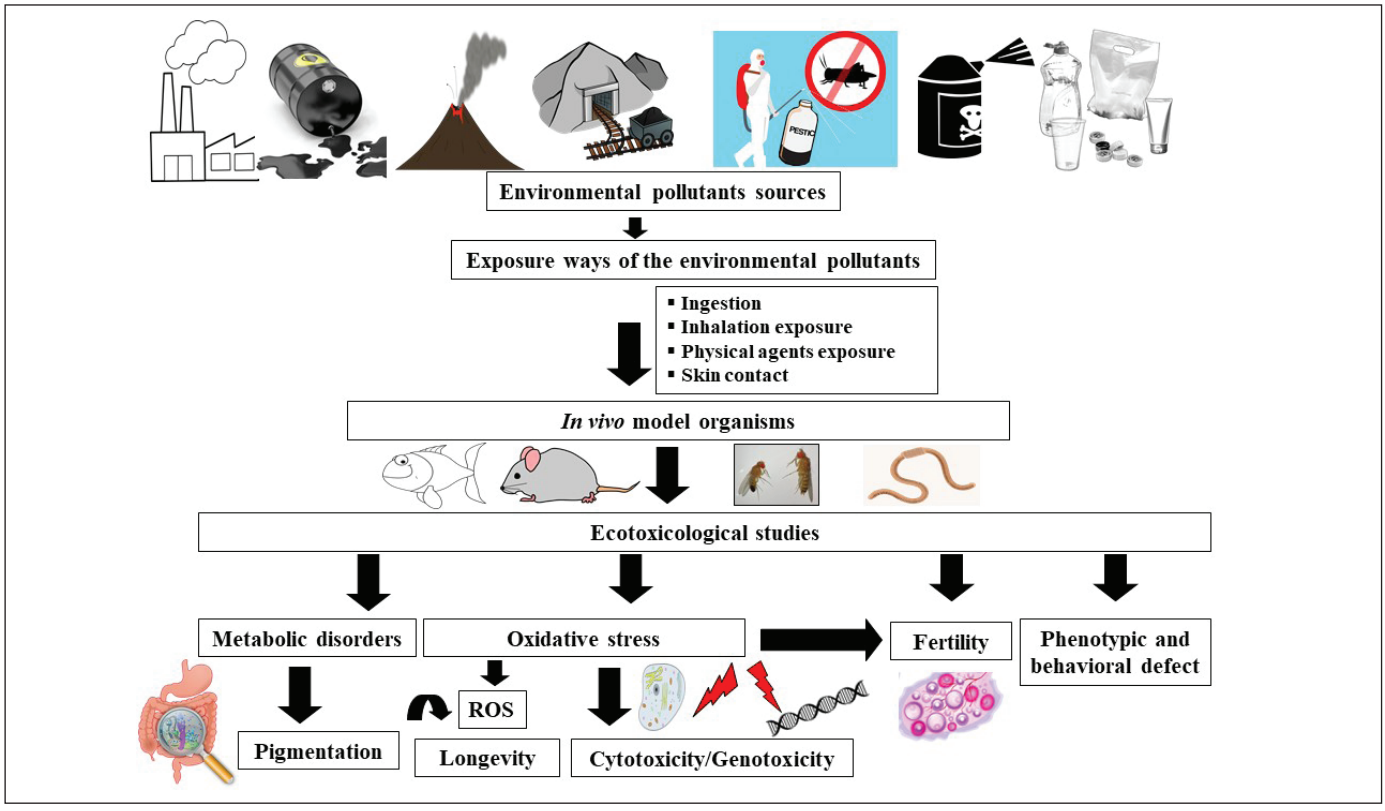

Figure 1. Evaluation of the biological/toxicological effects of environmental pollutants.

Table 1. Widespread risk assessment methods for mixtures of environmental pollutants and mixtures whose effects have been investigated in literature.

Used method	Details of the method	Investigated mixture samples	References
Adjusted or weight of evidence HI	HI incorporates pairwise assessment	Environmental mixture of different antibiotics	Marx et al. 2015
Physiologically-based pharmacokinetic Modelling (PBPK)	The PBPK aims to foresee pharmacokinetic interactions in mixtures at the tissue level	Mixtures of trihalomethanes	Niu et al. 2015
		Mixture of pesticide residues	de Sousa et al. 2014
Hazard Index (HI)	HI is formed by the addition of hazard quotients for each component chemical in a mixture	A mixture of different phthalates in food goods	Chang et al. 2014
		Pesticide mixtures on vegetable and/or fruit	Jensen et al. 2015
Toxic Equivalency Factor (TEF)	The most commonly used type of relative potency factor (RPF) method is the TEF	Binary mixtures of metals including cadmium, lead and copper	Gao et al. 2016
Whole mixture assessment	Whole mixture approaches utilize toxicity data in the form of the biological reaction to an whole mixture	Industrial wastewater containing phenol derivatives, preservatives and surfactants	Carbajo et al. 2015
		Mixture of toxic metals and gases	Sriram et al. 2015

most could damage certain species not considered pests. Only about 0.1% of the pesticides is thought to reach the target species after common application methods like aerial spraying, and the rest causes environmental contamination (Demir 2020b). According to reports, the total pesticide-related death toll passed 100,000 across the globe (WHO 2015), with children and women most vulnerable to their injurious effects. Therefore, we urgently need risk assessment methods to swiftly and efficiently investigate ecotoxicity and cytotoxicity of pesticides and nanopesticides. *D. melanogaster* might prove to be a promising *in vivo* model organism for the risk assessment and toxicological classification of nanopesticides (Demir 2020b).

3.2. Nanoparticles

Thanks to their dynamic structures, use of nanoparticles (NPs) has been gaining momentum across a wide range of sectors including electronics, cosmetics, medicine, and pharmaceuticals (Demir 2021c). Carbon-based NPs may have detrimental effects on human health as well as on the environment. However, the current literature contains limited research on the toxic and environmental effects from direct and indirect exposure to NPs. According to recent estimates, the production of NPs will reach 21,713 tons by 2020, which is sure to increase our exposure to such materials (Demir 2021c). While researchers often prefer to carry out *in vitro* studies for the detection of adverse effect of NPs, *in vivo* approaches seem to yield more relevant data for risk assessment. In that context, *D. melanogaster* is one of the most genetically and experimentally accessible model organisms used in biology as an *in vivo* model. Around 75% of the genes involved in human diseases have related sequences in *D. melanogaster*, fact that support its value as an effective model to study different human pathologies (Demir 2020b, 2021b).

3.3. Micro-Nanoplastics

Micro-nanoplastics (MNPLs) have recently attracted increasingly more attention, as they have been building up in the environment, multiplying our risk of exposure. A recent study has calculated that the oceans contain a total of 8.3 million pieces of MNPLs in every cubic meter of water (Brandon et al. 2020). Their abundant presence in nature and their tiny size facilitates their crossing cellular barriers and leads to potentially dangerous effects, thus calling for urgent risk assessment through *in vivo* toxicity studies.

Ingestion appears to be the primary route of exposure in many organisms, including aquatic animals, since MNPLs are usually found in their digestive tracts (Carbery et al. 2018). Besides, the tiny size of MNPLs allows their transfer through a range of tissues and organs (Alimba and Faggio 2019). Humans are mostly exposed to these plastics through consumption of seafood, however there is an alarmingly

limited amount of research to investigate potential toxicity of MNPLs in humans (Demir 2021b).

In an attempt to pinpoint environmental factors which could be considered to modulate risk of disease, researchers have proposed several biomarkers, including overproduction of ROS and RNS (Ghezzi 2020). Such biomarkers rank among the parameters used in toxicology studies looking into the effects of nanoplastics. A study exposing zebrafish larvae to nanoplastics detected ROS generation throughout the body, particularly in the head (Sökmen et al. 2019). The most common species of water flea, *Daphnia pulex,* was exposed to nanoplastics, which resulted in overproduction of ROS along with activation of the MAPK signalling pathway, impairing developmental and reproductive functions (Liu et al. 2020).

Another useful biomarker, genotoxicity plays a major role in the initiation and progression of different health problems, as DNA damage is known to cause genetic mutation, cancer, aging, and cell death (Barabadi et al. 2019). However, a rather limited number of studies has looked into genotoxic effects of MNPLs, and in those studies, hemocytes of aquatic organisms have been used as cell targets to detect any possible DNA damage. The researchers reported that MNPLs smaller than 100 μm induced DNA damage (Avio et al. 2015).

Although no contamination was thought to occur in Polar Regions, organic contaminants have been constantly detected in Antarctica since the early 1960s, when bases were set up for research purposes (Fidan and Ayar 2021). Increased fishing, touristic activity, and vehicle traffic appear to be the source of contamination in that region. A variety of sources such as waste disposal and ocean currents that carry microplastics (MPs) to Antarctica have been often shown as culprits (Fraser et al. 2016). Fidan and Ayar (2021) obtained water samples from three different stations in the Antarctic Peninsula to test the toxicity of water on *D. melanogaster*, reporting no adverse effects in flies, which could suggest that the contamination in that area has yet to reach the critical threshold.

Zhang et al. (2020) investigated possible interactions of MPs with other environmental and health hazards of common pollutants such as cadmium (Cd) and cadmium compounds in *D. melanogaster*, reporting that combined exposure to MPs with such materials could lead to gut damage in larvae and young flies, as well as impaired locomotor-behavioral function among adults. Besides, exposure to cadmium components were found to induce dramatic levels of gene-silencing in somatic cells. Their findings further support the dynamic potential of *D. melanogaster* as an experimental model in studies assessing possible effects of exposure to other contaminants mediated by MPs.

In another study, Jimenez-Guri et al. (2021) exposed *D. melanogaster* to MPs like polyvinyl chloride through food culture, and although they detected no effects on the size of adult flies nor on their immune capacity as compared to controls, the researchers found significant changes in the fecundity and male-to-female ratio.

Furthermore, Liu et al. (2022) explored the impact of MP exposure on *Drosophila*, discovering that such exposure caused a serious damage to adult fly's retina along with lower reproductive capacity. Such MP-induced phenotypes could be attributed to the altered gene expression caused by ligand-receptor interaction, phototransduction, endocytosis, and signaling pathways of Toll and immune deficiency (Imd).

Lastly, Demir (2021b) demonstrated that polystyrene microplastics (PSMPs) produced genotoxic effects in *Drosophila*. In this study, PSMPs induced genotoxic activity predominantly via initiation of somatic DNA recombination in a concentration-dependent manner.

3.4. Heavy Metals

A common group of environmental contaminants, heavy metals may prove to be toxic at low concentrations during the period from embryo formation to fetal development and birth (Gundacker and Hengstschläger 2012). For instance, Cd, both natural and industrial sources, has shown toxicity in animal tests. Another common contaminant, mercury (Hg) may enter the food chain through fish and seafood. Exposure to mercury during pregnancy could jeopardize the development of embryo and fetus through ROS formation and oxidative damage to macromolecules (Davidson et al. 2008). Research on mixtures of heavy metals, such as Cd, nickel, and lead along with arsenic and Cd has found increased mortality and immobilisation rates in *Daphnia magna* (Le et al. 2013). In the literature, heavy metal accumulation in the different organs and tissues and genetic damage in the blood samples were detected in the wild rodents (*Microtus guentheri*) collected from heavy metal contaminated areas (Turna Demir and Yavuz 2020).

Shilpa et al. (2021) studied the effects of heavy metal accumulation by exposing *D. melanogaster* to lead (Pb), an

environmental pollutant known to be associated with autism spectrum disorder (ASD), during the larval and adult stages. They reported that exposure to varying doses of Pb (0.2 to 0.8 mM) resulted in accumulation of Pb inside *Drosophila* brain and caused oxidative stress, which apparently led to ASD-specific symptoms like increased social spacing and grooming, as well as decreased memory, climbing, and learning. Their findings suggest that oxidative stress caused by Pb exposure from larval stage could lead to human ASD-specific behavioral patterns in *Drosophil*a. In a recent study, Yang et al. (2022) showed that Cd exposure caused DNA damage, oxidative stress via ROS, changes of apoptosis related genes in *Drosophila* larvae.

3.5. Pharmaceuticals

Wide range of pharmaceuticals are known to contaminate the environment which include non-steroidal anti-inflammatory drugs (NSAIDs) like high-dose aspirin, contraceptives, and antibiotics, whose mixture may prove to have highly toxic effects (Kümmerer 2009). In a toxicity study using *Daphnia magna,* a small planktonic crustacean, as a model organism, for example, exposure to mixture of NSAIDs was reported to cause significantly greater toxicity than individual medications (Cleuvers 2004). Another study reported that while exposure to individual drugs immobilized only 1% and 16% of *D. magna*, respectively, mixture of both drugs led to the immobilization of 95% (Cleuvers 2003), which represents a significantly greater toxicity than expected. Furthermore, a mixture of different antidepressant agents has been reported to result in higher deformity and mortality than single agents (Flaherty and Dodson 2005).

Antibiotics have also recently gained attention as emerging environmental pollutants associated with various harmful effects such as obesogenic properties. In a study, *D. melanogaster* were exposed to antibiotics, and the effects were measured on circadian rhythm, lipid metabolism, and microbiota. Circadian rhythm disorder was examined owing to its relationship with lipid metabolism and microbiota in obesity. Their results indicate that antibiotics could induce obesogenic effects as well as dysrhythmia and disturbances in lipid metabolism and microbiota (Yu et al. 2020).

3.6. Xenoestrogens

Considered as environmental chemical contaminants, xenoestrogens are known to act like endogenous hormones or impair endocrine processes, and they are abundant in most types of plastics, cosmetic products, packaging, drug coatings, and cosmetics (Klingmüller and Alléra 2011). Toxicity tests on mammals have revealed their toxic effects on reproductive system and embryonic health (Varayoud et al. 2011). Plasticizers, chemicals employed to produce more durable plastics, pharmaceuticals, and cosmetic products are also known for their potential threat to endocrine processes. Animal trials indicate that chronic exposure to plasticizers, or more commonly known as phthalates, exerts a serious impact on reproductive health, possibly leading to infertility (Kay et al. 2013). Previous research using cell cultures derived from monkey kidney demonstrates that 24-hour exposure to propylparaben, a type of preservative often found in cosmetic creams, shampoos, and lotions, results in increased oxidative DNA damage and induce cell death or cell cycle arrest (Martín et al. 2010).

Commonly used in wood paints applied to ships to prevent them from damage from marine organisms, tributyltin (TBT) may leak into aquatic environments, potentially causing pollution. In rats, exposure to tributyltin compounds after embryonic implantation has been found to increase the rate of malformation and loss in fetus. Another endocrine-disrupting type of xenoestrogen, triclosan (TCS) is usually present across several ecosystems due to its widespread use in consumer products. It has been reported that chronic exposure to triclosan could impair thyroid homeostasis in female rats and pubertal development in their offspring (Paul et al. 2012). Besides, it has been observed that rats exposed to triclosan during pregnancy suffer hypothyroxinemia at all stages of reproduction (Paul et al. 2012).

4. Other Pollutants

4.1. Indoor Air Pollutants

Because individuals in modern cultures spend the majority of their time indoors, air contaminants such as volatile organic compounds (VOCs) occur more frequently and at higher concentrations indoors than outdoors, indoor air quality has become a serious health concern. Formaldehyde, acetaldehyde, benzene, toluene, and perchloroethylene are examples of rather hazardous indoor VOCs, with toluene and formaldehyde most commonly found in workplaces and households. VOC exposure can cause a variety of health problems. However, the majority of research on indoor air pollution has relied on measuring VOCs in household indoor/outdoor habitats and human samples. Animal models for inhalation toxicity research are rather limited, with rodents being the most frequently used models, though animal testing on such mammals for screening indoor air pollution has its drawbacks (Wang et al. 2012).

D. melanogaster is one of the most actively researched alternative model organisms because it offers significant benefits over other model organisms, notably in the monitoring of air pollution. These flies have been reliably used to test the toxicity of compounds at various developmental stages, including heavy metals, industrial VOCs, and anesthetic agents (Rand 2010). Research has also been utilizing *Drosophila* to understand the mechanisms and genetics underlying susceptibility to ethanol intoxication, to measure variations in metabolic rate during exposure to gasoline components, to analyze olfactory avoidance behavior, and to describe neurotransmitter pathways influenced by volatile fungal toxins. Toluene and formaldehyde exposure causes toxicity, and gene expression in fruit flies, according to (Eom et al. 2017).

The majority of inhalation toxicity research has been carried out on rodent models. However, some researchers adapted such toxicity studies to *Drosophila*, an excellent model for studying the molecular processes of responses to air pollutants. Despite the lack of lungs, the airway systems of *Drosophila* can be compared to respiratory systems of mammals to serve as valuable model species for inhalation toxicity tests (Posgai et al. 2009).

Several studies conducted by Win-Shwe and Fujimaki et al. (Win-Shwe and Fujimaki 2010) have demonstrated that long-term exposure to low levels of toluene can disrupt neuroimmune crosstalk in the central nervous system. A genome-wide gene expression profile analysis on *Drosophila* revealed that toluene exposure altered numerous genes involved in biological regulation, defensive response, metabolic function, cell cycle, and circadian rhythms (Moskalev et al. 2014). However, no studies on the neurotoxic consequences of formaldehyde inhalation in *Drosophila* have been published. The microarray experiment by Eom et al. (2017) suggested that formaldehyde inhalation might also impair the *Drosophila* nervous system.

Recently, Weitekamp and Hofmann (2021) examined the impact of indoor air pollutants on locomotor behavior and possible changes in gene expression in *D. melanogaster*, by analyzing their aggressive behavior and courtship routines because this in-vivo model offers a series of advantages in such testing. They reported that exposure to toluene or formaldehyde via inhalation resulted in significant changes in gene expression comparable to those reported in higher vertebrates, which seriously altered pathways associated with stress and immune capacity.

Another study by Thimmegowda et al. (2020) also exposed *D. melanogaster* to air pollutants, detecting marked changes in locomotor behavior, survival, heart rate, hemocytes, along with changes in the expression of certain genes, particularly those known to play a role in immunity, stress, and metabolism. Macedo et al. (2022) reported that 1-octen-3-ol, known as mushroom alcohol, is a fungal VOC caused adverse mitochondrial activity and induced oxidant stress (nitric oxide (NO) and ROS) in *D. melanogaster*.

Since the body of *D. melanogaster* carries genes quite similar to those of humans - as much as 60% of *D. melanogaster*'s DNA is identical to that of humans - it stands out as an ideal experimental model to test potential health hazards of exposure to environmental pollutants. Besides, more than 70 percent of genetic material responsible for human diseases, including ASD, diabetes mellitus, and various cancers, displays a functional homolog in *D. melanogaster* (Lloyd and Taylor 2020). *Drosophila* was found to be a suitable model organism to determine the entry of various environmental pollutants into cells following exposure via inhalation and ingestion. Particularly, lethality and genotoxicity upon such exposure to environmental pollutants have been thoroughly studied for its potential adverse health consequences.

4.2. Haloalkanes

Over the recent decades, haloalkanes, a group of chemical compounds, have been utilized in industry as solvents, cleansers, anesthetics, and antiseptics. Some of these haloalkanes are thought to be present as pollutants in drinking water and chlorinated swimming pools. In general, haloalkanes are severely hepatotoxic and are proven human carcinogens. Carbon tetrachloride-induced hepatotoxicity has been widely researched throughout the years (Weber et al. 2003).

Lastly, Turna Demir (2022) noted that 1,4-Dioxane (DXN), which is a drinking water pollutant, caused toxic and genotoxic (mutagenic, recombinogenic, and DNA damage) effects in *D. melanogaster*. In addition, the nontoxic doses of DXN significantly induced oxidative stress, thermal sensivity, abnormal phenotypic alterations, and climbing behavior.

4.3. Hydrazines

Hydrazines are pollutants in the environment, are found in edible mushrooms, and are employed in medicine. Hydrazines are harmful in general, causing a range of toxic insults such as

liver toxicity, carcinogenicity, and mutagenicity. Hydralazine, the least hazardous of the hydrazines, causes DNA damage, severe forms of systemic lupus erythematosus, and a rise in lung tumor incidence in mice. Because of the importance of hydrazine derivatives as environmental and food pollutants, as well as their value in medicine, a substantial amount of study has been conducted to unravel the processes of toxicity of these molecules. According to research, when hydrazine derivatives are oxidized (mediated by metal ions, cytochrome P-450, and peroxidases), they produce free radicals that cause DNA oxidation and damage (Song et al. 2020). It has been postulated that substituted hydrazines can alkylate DNA by nucleophilic assault rather than free radical production. Nonetheless, DNA alkylation has been linked to carcinogenic events.

4.4. Smog

Smog, a serious health issue in industrialized countries, is frequent in big metropolitan areas, particularly during hot, bright weather. People over 65, as well as those with heart or lung problems, are especially vulnerable since the NOs, sulfur dioxide, and ozone included in this pollution are known to cause cellular damage through the formation of free radicals. Sulfite exposure has also been linked to allergic responses and bronchoconstriction in susceptible human groups. Ozone is a hazardous gas that is one of the principal pollutants in photochemical smog. It has been linked to lung inflammation and alveolar epithelial damage. It has been proposed that free radicals produced by the breakdown of secondary ozonides, a reaction result of ozone with unsaturated lipids, cause these effects (Wong 2017). Ozone has also been shown to cause DNA damage in cells via free radical-mediated processes (Wagner et al. 2021).

Eom et al. (2017) conducted an inhalation toxicity test using toluene and formaldehyde on *D. melanogaster* in order to investigate potential dangers of indoor air pollution. Their computational behavior analysis detected that such exposure impaired behavioral patterns of *Drosophila*. Inhalation of these compounds were found to cause severe toxicity besides significant changes in behavior and gene expression.

5. Toxic Effects of Environmental Pollutants on the Gut Microbiota

Dramatic changes in microbiota composition and/or optimal functions are have been linked to various metabolic and immune diseases, such as Crohn's disease (Darfeuille-Michaud et al. 2004), diabetes (Qin et al. 2012), allergic reactions (Ling et al. 2014), and colorectal cancer (Nakatsu et al. 2015).

Previous scientific work has found that human exposure to endocrine-disrupting chemicals to obesity, diabetes, and metabolic syndrome. However, it is unclear how the gastrointestinal (GI) microbiota and environmental chemicals interact and whether these interactions are relevant for human health. A recent review suggests that GI microbes might be playing a role in obesity and diabetes by altering the absorption, disposition, metabolism and excretion of environmental chemicals. Here we present the xenobiotic-metabolising capabilities of the GI microbiota and then address certain environmental contaminants identified as substrates of the GI microbiota. Even though several chemical reactions are attributed to GI microorganisms, limited number of enzyme families have so far been nominated for microbial metabolism of xenobiotics, which include sulfatases, β-glucuronidases, β-lyases, azoreductases, and nitroreductases (Claus et al. 2016).

Within the last decades, research into gut microbiota employed mostly utilized mammal test models to explore the role of interaction between microbiota, gut, and brain. However, *Drosophila* allows researchers to efficiently study the mechanisms whereby gut microbes affect the brain functions through observations of innate behaviors including courting routines, aggression, locomotor behavior, and temperature-related behavior (Chiang et al. 2022). Although there are a few studies to have reported no relationship between gut microbiota and courting routine in *Drosophila* (Jia et al. 2021); we should note that those studies employed different wild-types and different methods.

Li et al. (2022) reported exhaust gases from traffic significantly impaired life span and locomotion in *Drosophila*, with an increase in bacterial load in the guts upon exposure to such gases, which could induce microbiome depletion in guts, thus triggering changes in Imd and DUOX gene expression.

Different types of environmental pollutants are metabolized in the gastrointestinal flora. Polycyclic aromatic hydrocarbons (PAHs) are most commonly found in cigarette smoke, diesel exhaust, particulate matter (PMs) in urban air pollution and certain foods like smoked and grilled meat. Some have been classified as probable human carcinogens, and exposure to PAHs is linked to greater risk of lung and bladder cancer. In their study investigating their effects on human microbiota, Van de Wiele et al. (2005) found that PAH molecules were not oestrogenic, while the colonic digests displayed a significant oestrogenic activity, which suggests that the microorganisms within the human colon might bioactivate

PAHs by converting them into oestrogenic molecules. As for further toxicological potential, rat and human gut microbiota have been shown to be able to regenerate benzo(a)pyrene from its hepatic conjugate and reverse the endogenous detoxification (Claus et al. 2016).

Although certain synthetic pesticides, such as dichlorodiphenyltrichloroethane (DDT), have been banned in most developed countries, some of them widely persist as a source of contamination. Developed in the 1940s, DDT, the first modern synthetic insecticide, has been found to promote oestrogenic and antiandrogenic activity in specific tissues (Kim et al. 2014). Exposure to this compound increases the risk of developing several different types of cancers and metabolic diseases (Mrema et al. 2013). *In vivo* research carried out on rats, found that rats receiving DDT by stomach tube metabolized it into Dichlorodiphenyldichloroethane (DDD), which suggests possible involvement of the GI microbiota in DDT metabolism. Unfortunately, the role of the gut microbiota on pesticide metabolism has not been adequately investigated, and further research is warranted in this field.

Humans are primarily exposed to polychlorobiphenyls **(**PCBs) via ingestion of contaminated food, as well as inhalation and dermal contact. They have been associated with an increased risk of breast cancer (Negri et al. 2003), impaired reproductive function (Buck Louis 2014), and metabolic disruptions (Kim et al. 2014). The major metabolic route in mammals is hydroxylation followed by excretion, so the gut microbiota could be playing a crucial role in the formation of $MeSO_2$-PCBs. The metabolism of PCBs to $MeSO_2$-PCBs mediated by gut bacteria appears to be important from a toxicological perspective, because of their lipophilicity. Such compounds have been reported to accumulate in the human liver, lungs and adipose tissue, and may well be linked to lung dysfunction complaints occurring in poisoning due to food contamination incidents (Claus et al. 2016).

Some metals, including mercury, lead, cadmium, and arsenic, could be highly poisonous to living creatures. The highest toxicity is recorded in organic mercury compounds, and methylmercury (MeHg) is the main source of organic mercury in humans. Exposure to MeHg comes almost exclusively from consumption of seafood. Experiments that associated a decline or lack of gut microbiota with reduced faecal excretion of total mercury have been reported to confirm that demethylation for mercury is carried out by a microbial community in the gastrointestinal tract (Claus et al. 2016).

Another source of metal contamination, bismuth is a crystalline metal that can easily melt and so is often used in cosmetics, pharmaceuticals, alloys and pigments. Faeces samples collected from humans and isolated gut segments from rats have been found to metabolize bismuth into trimethyl bismuth, which is a toxic derivative of this metal (Michalke et al. 2008). Most notably, derivatives of other elements such as arsenic, antimony, tin and lead, have been reported to be produced at higher rates. Since volatile derivatives of metals often have greater toxicity than their inorganic precursors, such transformations mediated by gut microbiota might exert a significant impact on the host organism, however further *in vivo* studies are required to reach conclusive evidence in this respect.

Several chemical compounds are derived from benzene, which include phenol, toluene, and aniline. Nitrobenzene and its derivatives (NBDs) are highly toxic compounds released into the environment by human activity. Exposure to nitrobenzene is known to have a negative impact on the neurological system, cause liver necrosis, and degenerate seminiferous epithelium cells in animal trials. Studies on material in the cecum revealed that the gut microbiota continuously converted nitrobenzene to potentially hazardous NBDs. Antibiotic treatment has been reported to reduce reductive metabolite excretion and offer protection against methemoglobinemia induced by exposure to nitrobenzene. Therefore, the gut microbiota could be suggested to be main predictor of toxicity caused by NBDs (Claus et al. 2016).

Known for their vibrant orange and red hues, azo dyes are synthetic compounds accounting for more than half of global annual dye production, therefore much research has been focusing on the toxic potential of these compounds ever since they were associated with higher risk of cancer development as early as 1895. Certain azo dyes that are often utilized as food colourants have been reported to have very limited cytotoxic, mutagenic or carcinogenic effects, whereas others extensively used in industrial applications such as Sudan dyes are not permitted for food coloring due to their known toxicity. These solvent dyes are readily absorbed through the dermal route and human exposure mainly occurs via inhalation and ingestion. Recent and previous research offers unequivocal evidence that most azo dyes, once absorbed by the human body, can undergo microbiota-mediated decomposition and form carcinogenic amines (Feng et al. 2012). Some of the metabolites produced by gut microbiota in this way could be carcinogenic to

humans even though the parent azo dyes may not have an established potential to produce cancer (Feng et al. 2012).

A nitrogen-based compound often used to manufacture strong plastics and glues, melamine could at some instances be illegally used in protein content analyses as adding melamine to pet food or infant formula gives a falsely high protein level. As it is quite cheap and readily available, people in underdeveloped countries may see a financial opportunity despite its legal prohibition. The most common damage of melamine exposure in humans is kidney stones formed from melamine and uric acid, or from melamine and cyanuric acid. Zheng et al. (2013) reported that antibiotic therapy reduced the severity of melamine toxicity in rats and that melamine was converted to cyanuric acid in vitro by bacteria cultured from rat faeces. However, rats colonised with *Klebsiella terrigena* were found to suffer intensified renal damage induced by melamine (Zheng et al. 2013). The toxic effects of melamine on kidneys appears to have been mediated by the gut microbiota, depending on metabolic activities of microbial community in the gastrointestinal tract.

The health effects of artificial sweeteners have been a controversial issue. While some research reports benefits, others indicate greater risk of diabetes (Nettleton et al. 2009). Among the most commonly used artificial sweeteners in Europe is cyclamate, which is metabolised into cyclohexylamine, and this has been shown as the culprit that causes cancer, but such toxicity is still disputed in the scientific community. Cyclohexylamine has been identified as the primary urinary metabolite of ^{14}C-cyclamate in human and animal experiments. There is also plenty of evidence suggesting that gut microbiota is responsible for cyclamate metabolism. *In vitro* research has revealed that cyclamate is converted to cyclohexylamine by lower gut contents. An *in vivo* study gave lab rats different doses of cyclamate in drinking water for several months and found that they began to convert it into cyclohexylamine, however when they added antibiotics to water, they observed that this ability to convert cyclamate was lost. Several studies have made these observations in humans, offering conclusions that the gut microbiota could be solely responsible for metabolizing cyclamate (Claus et al. 2016). Demir et al. (2014) observed that the potential genotoxicity of different sweeteners (aspartame, acesulfame K, sucralose and saccharin) in *Drosophila* wing spot test and the Comet assay. They found that saccharin, acesulfame K and sucralose did not genotoxic effect in the used two assays. On the other hand, obtained results from aspartame in the Comet assay show a genotoxic effect.

Environmental pollutants can affect the metabolic activity or content of the gastrointestinal flora. Environmental factors, including lifestyle, eating habits and antibiotic use may easily affect the gut microbiota. The microbiome influences neurodevelopment, regulates behaviour and contributes to various neurological and neuropsychiatric disorders (Schretter et al. 2018). A range of environmental chemicals have also been shown to inhibit bacterial growth in the gut or to cause dysbiosis. Recent research indicates that both changes in microbial colonization during the perinatal period and exposure to environmental chemicals early in life can lead to impaired immune function (Menard et al. 2014). Thus, exposure to environmental contaminants might be affecting normal colonization of bacteria in the gastrointestinal tract, with effects on host physiology during later stages of life.

Furthermore, there is a general agreement that a balanced gut flora should not be defined as an idealized assembly of particular microbe populations, but rather that the microbes community should be able to perform a set of metabolic functions in collaboration with its host, though this series of metabolic functions has yet to be described. This is noteworthy since some xenobiotics may affect gastro intestinal microbiome composition without causing dysbiosis: as such, once fresh human faecal samples were incubated with antibiotics or host-targeted drugs, all host-targeted drugs resulted in significant changes of microbial gene expression, such as genes that control xenobiotic metabolism, despite having negligible short-term effects on microbial community structure (Maurice et al. 2013). A range of environmental contaminants rendered hazardous due to microbiome-mediated metabolism may be affected by such interactions, leading to alterations in their toxicity and risks. These compounds are also described in Table 2.

A common type of phosphonic acid, glyphosate is a popular pesticide moderately used to kill certain insects and weeds with relatively low toxicity to mammals. The lowest concentrations of glyphosate has been reported to inhibit the growth of *Enterococcus faecalis* isolated from excrements of farm animals (Krüger et al. 2013). Sensitivity to glyphosate appears to change from one strain of bacteria to another. While some bacteria such as certain Salmonella and Clostridium species are resistant to glyphosate, other good bacteria such as Enterococcus species, *Bacillus badius*, and *Bifidobacterium adolescentis* may be highly vulnerable to this compound (Shehata et al. 2013).

Table 2. Human exposure to environmental pollutants and their gut microbiota alteration effects.

Type of chemical	Exposure	Effects	Source material	References
Pesticides	- DDT; 0.29 μg/kg bw per day through nutrition - Chlorpyrifos; 0.01 to 0.14 μg/kg bw per day through nutrition	Dysbiosis at adulthood of rats was induced after perinatal exposure to chlorpyrifos (1 mg/kg bw per day).	- Food - Pollutants in air	Joly et al. 2013
Polychlorobiphenyls (PCBs)	- Non-dioxin-like (NDL)-PCBs: 2.71 ng/kg bw per day through nutrition	The PCBs mixture reduced the amount of many bacteria (especially Proteobacteria).	- Soil - Water sediments	Choi et al. 2013
PCBs	- Dioxin-like (DL)-PCBs: 0.29 pg toxic equivalency (TEQ) WHO_{98}/kg bw per day through nutrition	The PCBs mixture decreased the amount of many bacteria.	- Soil - Water sediments	Choi et al. 2013
Metals	- Cadmium 0.16 μg/kg bw per day through nutrition - Lead 0.2 μg/kg bw per day through nutrition - Arsenic 0.78 μg/kg bw per day through nutrition.	Cadmium, Lead and arsenic induced dysbiosis in mouse.	- Ubiquitous environmental contaminants	Fazeli et al. 2011, Lepage et al. 2011, Breton et al. 2013
Artificial sweeteners	- Acceptable daily intake (ADI) (Food and Drug Administration; FDA, US): Saccharin: 15 mg/kg bw, Aspartame: 50 mg/kg bw	Saccharin and aspartame induced dysbiosis in mouse and human.	- Food additives	Palmnäs et al. 2014, Suez et al. 2014
Other persistent organic pollutants (POPs) (e.g., Polychlorinated dibenzofurans; PCDFs)	- PCDD/Fs 0.176 pg TEQ WHO_{98}/kg bw per day through nutrition	2,3,7,8 Tetrachlorodibenzofuran (TCDF) induced dysbiosis in mouse.	- Environmental pollutants produced during industrial processes	Zhang et al. 2015
Nanoparticles (NPs)	- NPs can enter living organisms through inhalation, ingestion or by direct contact with the food web.	Silver: Reduction in the diversity of the gut microbiota of Ag NP-treated larvae in *D. melanogaster*	- Foods and food packaging, electronics, clothing, sunscreens, medical goods and cosmetics	Han et al. 2014

Another environmental contaminant, chlorpyrifos is an organophosphate insecticide widely used to protect crops against pests. An *in vitro* study on the human gastrointestinal tract inoculated with faeces from healthy humans examined the toxicity of chronic exposure to chlorpyrifos, as well as *in vivo* tests in rats exposed to the compound from fetal stage to 60 days of age (Joly et al. 2013). Exposure to chlorpyrifos induced dysbiosis of gut microbiota and decreased levels of friendly bacteria. As far as we are aware, this is the only study to investigate the impact of perinatal exposure to low doses of environmental contaminants on gut microbiota.

A study where rats were exposed to cadmium through drinking water reported a sharp reduction in the populations of all microbial communities (Fazeli et al. 2011). Furthermore, chronic exposure to cadmium and lead in rats resulted in specific changes in the composition of microbiota. Animals that were exposed to heavy metals through food have been found to have lower count of friendly bacteria as compared to controls, but such exposures led to no toxicity and or hepatotoxicity, as well as no significant changes in behaviour, body weights, food intake, stool consistency or gut motility (Breton et al. 2013). Nevertheless, low count of friendly bacteria the family Lachnospiraceae have been associated with intestinal inflammation and predisposition to colitis (Lepage et al. 2011). Another study exposing rats to arsenic for 4 weeks reported a significant reduction in Firmicutes population, but not in Bacteroidetes numbers. Such changes in gut microbiota have been associated with metabolic activity alterations as evidenced by steep variation in a range of microbial co-metabolites detected in excrement samples of model organisms (Lu et al. 2014).

Common artificial sweeteners are known to contain compounds such as aspartame, sucralose and saccharin, which have been shown to cause significant disruption to the microbiota homeostasis and disproportionate microflora, changes in metabolic activities of animals and humans. Studies on mice exposed to sucralose have reported increased faecal counts of total anaerobes, with decline in the count of friendly bacteria (Abou-Donia et al. 2008). Likewise, another study treating mice with low doses of aspartame in drinking water detected a rise in total bacteria and in *Enterobacteriaceae* and *Clostridium leptum* counts (Palmnäs et al. 2014), as well as higher serum propionate and elevated fasting glucose values. A human study involving five to seven days of saccharin consumption reported that the majority of participants had significantly poorer glycemic response and marked changes in their gut microbiota during the study (Suez et al. 2014). Overall, these findings points to the fact that each individual responds to artificial sweetener exposure differently, possibly resulting from the variations in their gut microbiota composition.

6. Conclusions

Drosophila can dynamically meet requirements of toxicity studies looking into environmental contaminants as it allows optimization of pathway-specific screening, facilitates rapid testing of samples at cellular or molecular levels, and rapid identification of genes responsible for interactions with environmental contaminants. Its ability to reflect the true interactions between DNA and environment might shed light on the toxicity mechanisms of certain substances. Even though *in vivo* research carried out with other non-mammalian models like zebrafish (*Danio rerio*) may offer some solution, *Drosophila* appears to be superior at many levels, and it allows researchers to process numerous samples in a short time and to identify antibodies and genes regulating certain pathways at much lower costs, yielding valuable data on vital parameters like longevity, survival, mortality, and mutagenicity (Demir 2020a, 2022). Also, ethical rules (3R: Replacement, Reduction, and Refinement) concerned with vertebrate animals do not apply to *Drosophila* (Jennings 2011). Lastly, the toolbox of *Drosophila* which allows easy genetic manipulation is unique among different model organisms such as *D. rerio, Caenorhabditis elegans* (worm), and *Daphnia magna* (Mohr et al. 2014).

This review summarizes our current knowledge from previous *in vitro* and *in vivo* research that scrutinizes the impact of several environmental contaminants on various mammals and non-target model organisms at several genetic, cellular, and molecular levels, as well as possible mechanisms of their toxicity. Although *in vitro* approaches are the most used for testing the potential harmful effects of environmental pollutants in different properties, *in vivo* studies can provide much more important information complementing *in vitro* data. *D. melanogaster* has become an ideal model organism for the risk assessment and ecotoxicological classification of environmental pollutants on the environment and human health. *D. melanogaster* can be used as an ideal model organism for the risk assessment and toxicological classification of environmental contaminants. In this context, *D. melanogaster* can prove an ideal model organism for their risk assessment and toxicological classification, since it shares an outstanding amount of physiological features in molecular pathways with humans. The new research field

known as Drosophotoxicology may offer valuable data on the hazards of environmental contaminants. Collaboration with this valuable species of fruit fly has played an integral part in numerous Nobel-winning studies, and it will definitely serve as a versatile toolbox to further our understanding of environmental contaminants and their effects on living organisms.

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