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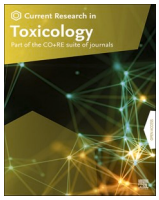

The utility of alternative models in particulate matter air pollution toxicology

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ABSTRACT

Exposure to particulate matter (PM) air pollution increases risk of adverse human health effects. As more attention is brought to bear on the problem of PM, traditional mammalian *in vivo* models struggle to keep up with the risk assessment challenges posed by the countless number of unique PM samples across air sheds with limited or no toxicity information. This review examines the utility of three higher throughput, alternative, *in vivo* animal models in PM toxicity research: *Danio rerio* (zebrafish), *Caenorhabditis elegans* (nematode), and *Drosophila melanogaster* (fruit fly). These model organisms vary in basic biology, ease of handling, methods of exposure to PM, number and types of available assays, and the degree to which they mirror human biology and responsiveness, among other differences. The use of these models in PM research dates back over a decade, with assessments of the toxicity of various PM sources including traffic-related combustion emissions, wildland fire smoke, and coal fly ash. This article reviews the use of these alternative model organisms in PM toxicity studies, their biology, the various assays developed, endpoints measured, their strengths and limitations, as well as their potential role in PM toxicity assessment and mechanistic research going forward.

1. Introduction

Particulate matter (PM) air pollution is ubiquitous and continues to exact a substantial burden on public health. More deaths in the U.S.A were attributable to elevated fine PM in 2005 than deaths due to accidents or influenza (Fann et al., 2012). These effects are characterized largely by the worsening of respiratory and cardiovascular disease (Pope et al., 2004) and are influenced by both the size and composition of PM. Coarse PM (PM_{10}, aerodynamic diameter < 10 μm) often originating from natural sources (e.g., pollens, crustal material,), as well as brake and tire wear, deposits mainly in the upper airways (Cen et al., 2020). Fine ($PM_{2.5}$, diameter < 2.5 μm) and ultrafine PM ($PM_{0.1}$, diameter $<$ 0.1 μm,) mainly originating from the combustion of fossil fuel and high temperature industrial processes, penetrate deep into the alveolar region, increasing the likelihood for lower lung and extra-pulmonary effects. PM air pollution of all size ranges is a complex mixture, consisting of elemental carbon, ionic species, metals and organic compounds, the composition of which varies according to local sources, long-range transport, photochemistry, and wind conditions (Brook et al., 2010). The spatiotemporal heterogeneity of PM across air sheds results in countless unique PM samples with only sparse information on toxicity and mode of action, which impedes risk assessment.

For decades, toxicological studies have bolstered epidemiological findings of PM health effects by demonstrating biological plausibility. Mammalian *in vivo* approaches, most often rodent models, usually involve in-life inhalation or intra-airway exposure, and while closely modeling human whole organism physiology and route of exposure, are relatively expensive and have low throughput. As such, traditional testing in intact rodent models is not ideal for screening the relative toxicity of large numbers of PM samples or quickly assessing the impacts of compositional differences on mode(s) of action. Higher throughput *in vitro* methods that culture human respiratory epithelial cells fail to recapitulate the complexities of metabolism and the interplay between cells and tissues in intact organisms. Over the last decade, alternative model organisms such as the zebrafish, nematode, and fruit fly have been adopted in routine toxicity studies in large part because they are fully intact *in vivo* models and offer the logistical ease and throughput of *in vitro* assays (Gross et al., 2017; Rudich et al., 2020; Song et al., 2020). The specific applicability of such models in air pollution-derived particulate matter research has not been systematically examined.

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Table 1
Characteristics of alternative in vivo models and PM study attributes.

	Danio rerio	*Caenorhabditis elegans*	*Drosophila melanogaster*
Lifespan	2–3 years, reproductive maturity in 3 months	18–20 days	90 days
Age range studied	Fertilization to 7 dpf	1–2 days, transgenerational exposure	Lifespan
Exposure Type	Immersion, Injection	Immersion, feeding	Aerosol, injection, feeding
Common Endpoints	Embryotoxicity, mechanistic pathways, developmental	Toxicity, mechanistic pathways, transgenerational effects	Lifespan assays, genotoxicity, mechanistic pathways

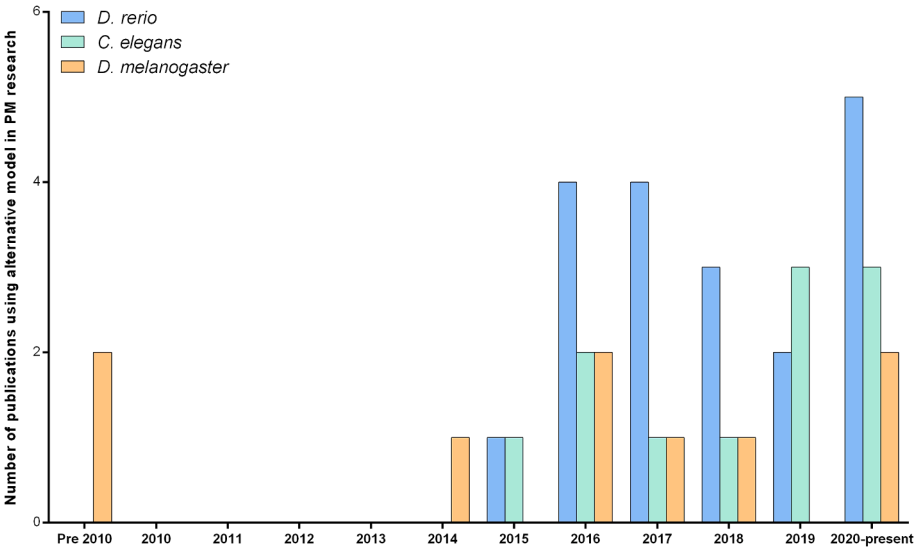

Fig. 1. Total number of publications per year involving use of alternative *in vivo* models (i.e., *D. rerio, C. elegans, and D. melanogaster*) in particulate matter research. The studies referenced are limited to anthropogenic sources of particulate matter.

Table 2
Inclusion & Exclusion criteria for studies cited in Fig. 1.

Inclusion Criteria	Exclusion Criteria
• The search terms included "*D. rerio* + Particulate Matter", "zebrafish + Particulate Matter"; "*C. elegans* + Particulate Matter", "nematode + Particulate Matter"; "*D. melanogaster* + Particulate Matter"	• Studies focused on engineered nanomaterial and microplastics were excluded
• Literature review was restricted to peer-reviewed studies on anthropogenic air pollution-derived particulate matter	• This review only cited peer-reviewed studies that were published in English
• Conducted on 08/25/2021	• Systemic reviews and editorials excluded from the literature review

Alternative *in vivo* models are lower order models and are compliant with the guidance laid out by the 3 R's principle and various regulatory bodies informing the use of animals in scientific research (Freires et al., 2017). In fact, the National Research Council (NRC) recommends toxicity testing reduce the number of animals tested, use non-mammalian species to reduce cost and time of testing, and use high and medium throughput assays that measure simple processes that can be measured rapidly (NRC, 2007). PM research, however, poses unique challenges that complicate adoption of alternative models, namely the complex composition of PM, the mimicking of the route of exposure, and cross-species translatability. The goal of this review is to describe the more popular alternative *in vivo* models, including zebrafish, *C. elegans*, and *Drosophila*, examine their current use in PM air pollution studies, and provide a path forward regarding their use in new studies. While the *in vivo* models described vary considerably in their biology, they have been used to study a variety of endpoints after various routes of exposure in PM toxicity research. (Table 1). Although larval zebrafish are not considered genuine *in vivo* models in the European Union (Bertelli et al., 2017), they are regulated as such in the United States (Cassar et al., 2020). For this reason, zebrafish across all stages of life will be described as *in vivo* models in this review. The use of these models in PM toxicity research has steadily increased over the years (Fig. 1). Table 2 lists inclusion and exclusion criteria for the PM studies cited in this review.

2. *Danio rerio*

2.1. Characteristics

The zebrafish (*Danio rerio*), a small freshwater fish native to Southeast Asia, is a widely used animal model growing in popularity as an alternative to *in vivo* mammalian models. Zebrafish can grow up to 4 cm long and are named for the blue stripes that run along the length of their body (Stednitz and Washbourne, 2020). Zebrafish are sexually dimorphic - males are narrower, torpedo shaped, and yellow-tinged (Nasiadka and Clark, 2012). Females of the species tend to be plumper due to the eggs they carry (Nasiadka and Clark, 2012). The sex differentiation of zebrafish is determined not by XY chromosomes, but by genetic factors on a polygenic basis (Santos et al., 2017). The zebrafish has a typical lifespan of around 3 years and can live up to 5 years (Ogura et al., 2021). When zebrafish are bred in the laboratory, a mating pair is typically picked by a researcher the afternoon before and placed into a mating tank, consisting of a tank with a second inset layer with a perforated bottom and a divider separating the fish until the morning (Nasiadka and Clark, 2012). When morning arrives, the divider is removed, and zebrafish will begin to spawn. At this point the fish can be returned to their original tank in the laboratory fish facility. Zebrafish produce hundreds of offspring per week, develop rapidly (5–6 days, depending on rearing temperature), and are ready to produce a new generation of offspring in 3 months (Singleman and Holtzman, 2014). Zebrafish are typically reared at 25–28 °C (Kimmel et al., 1995). Zebrafish embryos

are translucent and encased in an optically clear chorion, enabling convenient imaging and morphological assessment. Zebrafish can absorb chemicals from their environment through their epidermis (Morikane et al., 2020). Simple morphological measurements such as visualizing the heart using light microscopy are also possible at this stage (Martin et al., 2019).

There is a high degree of conservation between humans and zebrafish in terms of biological complexity. As for genetics, the total length of the zebrafish genome is 1,412,646,843 base pairs, within which are 26,206 protein-coding genes (Howe et al., 2013). Seventy percent of human genes have at least one zebrafish counterpart and have many homologues with disease causing genes in humans (Howe et al., 2013). As a matter of fact, 84% of genes known to cause disease in humans have a homologue in zebrafish (Howe et al., 2013). Well-characterized mutant strains of zebrafish are available for use in research regarding the mechanisms of disease and genetic function. Many of the zebrafish organs develop and function similarly to humans, such as a liver, which can activate and detoxify chemicals (Goessling and Sadler, 2015), thyroid tissue that controls development (Porazzi et al., 2009), and a well-studied neuronal network which can perform complex sensory, motor, and cognitive functions during larval stages (Ma et al., 2019). Zebrafish possess all the classic sensory systems: taste, tactile, smell, balance, vision, and hearing (Moorman, 2001). Their small size allows embryos to develop in traditional 96 well plates up to about 7 days of age, and the quick generation time facilitates experiments on a larger and faster scale than traditional mammalian *in vivo* models. Zebrafish have well-understood and observable developmental behaviors (Stednitz and Washbourne, 2020) and a diurnal sleep cycle like mammals (Leung et al., 2019). The characteristics of the zebrafish make them well suited for studies in immunology, regeneration, developmental biology, and the genetic pathways of disease (Poss, 2002; Song et al., 2020; Trede et al., 2004). Moreover, the easy handling, chemical permeability, and optical transparency of the zebrafish larva facilitate their use in routine toxicity screening.

Zebrafish larval skin epithelial responses have been previously suggested to be predictive of mammalian lung epithelial responses (McLeish et al., 2010). In addition, zebrafish gills, which have structural similarity and serve similar gas exchange roles as mammalian alveolar epithelium, mimic the inflammatory responses to air pollutants exhibited in human respiratory tissue (Progatzky et al., 2016). Embryonic and larval zebrafish differ in their mode of respiration from adult zebrafish, relying on passive diffusion of O_2 through the epidermis. They can do so because their large surface to volume area allows passive diffusion alone to supply O_2 to the mitochondria (Hughes et al., 2019). In addition, zebrafish skin and mammalian lung epithelium are sensitive to many of the same irritants including the aldehyde acrolein (Stevens et al., 2018). Zebrafish skin expresses the highly conserved transient receptor potential cation channel TRPA1, which mediates chemo-sensation to exogenous and endogenous agents (Stevens et al., 2018) including chemicals implicated in causing oxidative stress such as acrolein (Takahashi and Mori, 2011). We have previously demonstrated that TRPA1 mediates diesel exhaust particulate (DEP)-induced acute locomotor responses in zebrafish (Stevens et al., 2018).

2.2. PM research

The sources of PM used in zebrafish studies are diverse – they range from ambient PM in urban/rural areas and samples of wildfire smoke, to pollutants like coal and diesel exhaust (Duan et al., 2017; Caballero-Gallardo et al., 2018; Zhang et al., 2018; Martin et al., 2019; Cen et al., 2020). These studies provide examples of the variety of PMs researchers have used in toxicity assessments using the zebrafish model and include total particulate matter (TPM), extractable organic material (EOM), and fractionated PM (Stevens et al., 2018). The PM samples used in these studies were collected using glass-fiber filters or quartz films, which allowed for PM to be extracted into solvent through standard extraction methods such as sonication (Zhang et al., 2016). PM used in zebrafish studies is often extracted using water or organic solvents such as dichloromethane, methanol, or dimethyl sulfoxide (DMSO). Typical concentrations of PM in zebrafish studies ranged from 0.001 μg/ml to 2.5 mg/ml (Barnhill et al., 2020; Caballero-Gallardo et al., 2018; Stevens et al., 2018). In most studies the extracted material has been applied to larval zebrafish through immersion or microinjection into embryos (Kim et al., 2015). Immersion dosing of zebrafish raises the question of whether the results are due to the interaction of PM components with the skin or are due to internalization of PM chemical constituents. Because of the potential for chemical uptake, the relative contribution of chemical internalization in the elicitation of responses is unclear. Importantly, many organic components of PM, particularly hydrophobic chemicals such as polycyclic aromatic hydrocarbons (PAH) shown to trigger inflammatory responses when internalized (Bai and van Eeden, 2013), are absorbed by zebrafish (Kuhnert et al., 2013).

Zebrafish studies have been useful in identifying the relative toxicity of various PM sources. For example, we previously reported (Martin et al., 2021) that pine biomass induced a stronger irritant response in a locomotor assay at a given concentration than other wildlandfire-related PMs, including eucalyptus, pine needles, red oak, and peat samples. Also, we found that irritant responses significantly correlated with PAH content, but not with organic carbon or methoxyphenols (Martin et al., 2021). Roper and coworkers (2019) compared the impacts of different extraction methods on chemical composition of PM extracts and developmental toxicity in zebrafish. They found that extraction method affected elemental composition and PAH content in the extracts and influenced various endpoints including mortality (Roper et al., 2019). Zebrafish studies have also been used to identify chemical constituents that cause toxicity. For example, we previously demonstrated that polar and moderately polar PAHs (i.e., nitro and oxy-PAH constituents) drove toxicity of an organic extract of compressor-generated DEP at concentration ranges from 0.125 to 40 μg EOM/ml (Stevens et al., 2018). This is consistent with findings indicating that nitro-PAHs may be important causative agents of toxicity of traffic-derived $PM_{2.5}$ in human lung bronchial epithelial cell lines (Oh et al., 2011). Exposure to extracted organic matter from DEP at concentrations of 10–25 μg/ml has also been found to disrupt autophagy in zebrafish, resulting in behavioral deficits and a significant decrease in neuron number (Barnhill et al., 2020). Similarly, human bronchial epithelial and umbilical vein endothelial cells exposed to DEP had significantly reduced cell viability through induction of the autophagic pathway (Colasanti et al., 2018; Wang et al., 2017a). Moreover, exposure of zebrafish to urban-derived PM_{10} caused developmental neurotoxicity and dysfunction of dopaminergic neurons in zebrafish embryos (Zhang et al., 2021). DEP exposure induces neuroinflammation, oxidative stress, and neurodegenerative-related tau overexpression and regulation by autophagy in human neuroblastoma cells (Bai et al., 2018).

Investigators have also examined $PM_{2.5}$'s potential for developmental toxicity (Manjunatha et al., 2021). Acute and developmental toxicity assays of zebrafish exposed to PM can be run in a variety of vessels, including glass petri dishes, and welled plates of various sizes (24 to 96-well). To test developmental impacts, Cen et al. (2020) exposed 4-hour post fertilization zebrafish embryos to varying concentrations of PM and studied at 24, 48, 72, 96, and 120 h after PM administration. They found that PM_{10} induced an increase in levels of ROS present and significant increases in the expression levels of endoplasmic reticulum stress (ERS) signaling pathway factors and Nrf2 signaling pathway factors (Cen et al., 2020). In another study, the EOM of $PM_{2.5}$ from urban areas impaired development and/or function of the heart, liver, and neurons of zebrafish. $PM_{2.5}$ at concentrations of 25–400 ug/mL was also found to cause pericardial edema, decreases in axonal integrity, and morphological defects associated with hepatocyte injury in zebrafish in a dose and time-dependent manner (Duan et al., 2017). These findings are consistent with the growing body of evidence linking gestational exposure to PM to adverse outcomes in newborns and organ

Table 3
PM Studies in *D. rerio*.

Study/ Reference	PM type	Exposure method	Concentrations	Major findings
Duan et al., 2017	Urban $PM_{2.5}$	Immersion of embryos in solution containing $PM_{2.5}$	25, 50, 100, 200, 400 μg/mL	Cardiovascular toxicity, hepatotoxicity and neurotoxicity in zebrafish
Caballero-Gallardo et al., 2018	Coal dust particulate	Immersion of embryos in solution containing extract	0.1, 1, 10, 100, or 1000 μg/mL	Gene ontology analysis identified alterations to multiple signalling pathways
Zhang et al., 2018	Urban $PM_{2.5}$	Immersion of embryos in solution containing $PM_{2.5}$ extract	200, 300, 400, 500, 600 and 800 μg/mL	Reduced locomotion, developmental toxicity induced by inflammation and autophagy pathways
Zhang et al., 2021	Urban PM_{10}	Immersion of embryos/larvae in PM_{10} solution	25, 50, 100, 200, and 400 μg/mL	Reduced locomotion, evidence of neurodevelopmental toxicity through disruption in the development of dopaminergic neurons
Martin et al., 2021	Biomass smoke condensates, i.e. extractable organic material (EOM)	Immersion of embryos/larvae in solution containing EOM	0.3, 0.96, 3.0, 9.6 or 30 μg EOM/ml	Fuel type impacted irritant response in a locomotion assay; irritant responses correlated with (PAH) content
Cen et al., 2020	Urban PM_{10}	Immersion of embryos in solution containing PM_{10}	25, 50, 100, 200, and 400 μg/mL	Cardiovascular developmental toxicity in zebrafish embryos and larvae via the ERS, Nrf2 and Wnt pathways
Zhang et al., 2016	Urban $PM_{2.5}$	Immersion of embryos in solution containing EOM	0.2, 1, 5 mg/L	Crosstalk from AhR activation by EOM exposure may repress wnt/β-catenin signaling, leading to cardiac developmental toxicity
Barnhill et al., 2020	Diesel exhaust particulate (DEP) extract	Immersion of embryos in solution containing DEP	10–25 μg/ml	Behavioral deficits and a decrease in neuron number, partly attributed to autophagic flux
Stevens et al., 2018	Extractable organic matter of a compressor-generated diesel exhaust PM (C-DEP)	Embryos plated into sigle wells of a 96 well plate; larvae immersed in solution containing C-DEP	0.125–40 μg/ml	Concentration-dependent locomotor responses; TRPA1 antagonist blocked locomotor responses to extract
Kim et al., 2015	Urban $PM_{2.5}$	Microinjection of embryos & immersion of embryos in water containing $PM_{2.5}$	Microinjection: 30 ppm Immersion: 0.0003 to 300 ppm	Embryonic toxicity via aggregation and proteolytic degradation of serum lipoproteins; injection induced mortality/imparied skeletal development
Roper et al., 2019	Urban $PM_{2.5}$	Immersion of embryos in solution containing $PM_{2.5}$	200 μg/mL	Different methods of extracting $PM_{2.5}$ from filters changed the concentrations of elements and PAHs, leading to difference in mortality in zebrafish
Manjunatha et al., 2021	Diesel exhaust particulate (DEP) extract	Immersion of embryos/larvae in solution containing DEP	0, 5, 10, 15, 20, 25, 50, 75 and 100 μg/mL	Exposed embryos exhibited mortality, hatching delays, pericadial edema, disruption of the vascular system/ liver, and inhibition of motor neuron growth
Massarsky et al., 2016	Cigarette-derived Total Particulate Matter (TPM)	Immersion of embryos in solution containing TPM	1.43, 7.1, and 14.3 μg TPM/mL	The AHR pathway was induced in response to TPM exposure
Ren et al., 2020	EOM extracted from urban $PM_{2.5}$	Immersion of embryos in solution containing extracts	5 mg/L	Embryos dosed with resveratrol were protected against PM2.5 induced heart deformities through oxidative stress inhibition
Jiang et al., 2019	EOM extracted from urban $PM_{2.5}$	Immersion of embryos in solution containing extracts	5 mg/L	Embryos dosed with folic acid were protected against $PM_{2.5}$ induced heart deformities by attenuating DNA methylation and gene expression changes
Dai et al., 2021	Urban $PM_{2.5}$ collected from Beijing, China	Immersion of embryos/larvae in solution containing extracts	10 μg/mL	Treatment with various concentrations of fucoxanthin reduced expression levels of factors involved in inflammatory responses in $PM_{2.5}$ exposed ZF embryos
Mesquita et al., 2016	Environmental samples of atmospheric TPM from the Mediterranean and Black Seas	Immersion of embryos in solution containing extracts	N/A	No increases in mortality observed, up-regulation of mRNA expression of cyp1a, fos and development-related genes correlating to PAH content and dioxin-like activity of extracts
Mesquita et al., 2017	Urban $PM_{2.5}$	Immersion of embryos in solution containing extracts	N/A	No increases in mortality observed, toxic potential of PM1 strongly depends on the emission sources and on the process of ageing from primary to secondary organic aerosols
Babić et al., 2021	Biomass combustion byproducts from Amazon wildfires, specifically methoxyphenols	Immersion of embryos in solution containing methoxyphenol and byproduct extracts	1.17 up to 300.00 mg/L	Methoxyphenols inhibit tyrosinase, lipoxygenase, and carbonic anhydrase, consequently altering ZF embryonic development

Studies listed in order in which they appear in manuscript.

system-wide effects in children (Johnson et al., 2021).

Beyond characterizing toxicity, studies have also focused on the mechanisms of PM's effects including use of interventional approaches to mitigate the effects of PM. For example, the oxidative stress and the aryl hydrocarbon receptor (AHR) pathways have been found to separately play a role in the induction of congenital heart defects in zebrafish development from cigarette smoke (Massarsky et al., 2016) and extractable organic matter from urban $PM_{2.5}$ (Zhang et al., 2016), respectively. Other studies have focused on the protective effects of treatments in minimizing PM-induced toxicity in zebrafish. For example, Ren et al. (2020) studied the mechanism by which resveratrol protects against $PM_{2.5}$-induced AHR mediated reactive oxygen species (ROS) generation and subsequent heart defects. Yet more studies have focused on the protective effects of folic acid and fucoxanthin, a carotenoid in PM-induced inflammation, oxidative stress, and cell death in zebrafish (Jiang et al., 2019; Dai et al., 2021). Table 3 is a summary of previous PM studies involving zebrafish.

2.3. Limitations

PM immersion studies in zebrafish mimic the natural route of exposure to PM in aquatic organisms that may result from watershed runoff, ash deposits, or other forms of contamination (Mesquita et al., 2016; Mesquita et al., 2017; Babić et al., 2021) making it a useful model for screening the ecotoxicological effects of PM and other chemicals. However, PM exposure via immersion and injection contrasts with the

Table 4
PM Studies in *C. elegans*.

Study/ Reference	PM type	Exposure method	Concentrations	Major findings
Sun et al., 2015	Coal combustion related PM2.5	$PM_{2.5}$ solutions were prepared with K-medium	0.01, 0.1, 1, 10, and 100 mg/L	Oxidative stress and abnormal defecation behavior
Wu et al., 2017	Coal combustion related PM2.5	$PM_{2.5}$ solutions were prepared with K-medium	1 mg/L of PM2.5	miRNA dysregulation
Wang et al., 2019	Diesel exhaust PM	$PM_{2.5}$ solutions were prepared with K-medium	0.01, 0.1, 1 μg/mL	Reproduction deficits; decreased brood size and germ cell apoptosis
Volta et al., 2020	Brake system – derived PM	PM2.5 solutions were prepared with K-medium and exposed for 24 h	1, 10, 100, 500, 1000 mg/L	No toxic effects observed
Zhao et al., 2019	Ambient rural PM2.5	$PM_{2.5}$ solutions were prepared with K-medium	0.1, 1, 10, and 100 mg/L	Unfolded protein response and shortened lifespan through induction of oxidative stress
Yang et al., 2016	Ambient urban PM2.5	$PM_{2.5}$ solutions were prepared with K-medium; acute exposures dosed for 24 hrs while prolonged exposure was performed from L1-larvae to young adults (4 days)	0.1–10 mg	Decreased locomotion behavior, induction of intestinal ROS production, and induced expression of gene (mtl-1 and mtl-2) encoded metallothioneins
Chung et al., 2020	Traffic-related-air-pollutant PM2.5	$PM_{2.5}$ solutions were prepared with K-medium	1.61, 16.1, 161, 1610 g/L 0.743, 7.43, 74.3, 743 g/L 1.29, 12.9, 129, 1290 g/L	Shorter lifespan and brood size in a dose-dependent fashion
Yan et al., 2021	Diesel exhaust PM	PM solutions were prepared with K-medium	0, 0.01, 0.1, 1, 10 μg/mL	Impeded locomotion, dopaminergic function disorders, and upregulation of *hsp-16.2*

Studies listed in order in which they appear in manuscript.

primary route of exposure to PM in humans, i.e., inhalation. This is a clear limitation of the zebrafish model compared to studies in rodents, which are routinely exposed to inhaled PM aerosols and have a respiratory system analogous to humans (Lee et al., 2021). Although the differences in route of exposure – i.e. inhalation in humans vs. immersion dosing in zebrafish - are a challenge to reconcile, the zebrafish model may be used to screen for the potential to elicit mammalian toxicity. Importantly, potency determinations derived from irritant locomotor responses to wildland fire-related PM in zebrafish (Martin et al., 2021) were consistent with separate assessments of the same PM samples in mouse pulmonary toxicity studies that measured pulmonary neutrophil influx (Kim et al., 2019).

3. *Caenorhabditis elegans*

3.1. Characteristics

The nematode worm *Caenorhabditis elegans* is an exceedingly less complex organism compared to mammals, and yet has provided significant scientific insight. *C. elegans* is a multicellular organism that has become popular in nearly every field of biological study since its introduction to the laboratory. It is an appealing model due to the ease of rearing in the laboratory, the large number of offspring, and the robust body of research using the model. *C. elegans* is about 1 mm long at full size, and in the laboratory is usually fed a diet of *E. coli* plated on agar (Kiyama e al., 2012). *C. elegans* is typically grown at 20 °C in the laboratory, but it can also tolerate temperatures from 15 to 25 °C (Gómez-Orte et al., 2018). Its size allows it to be studied using simple microscopy using sub-stage illumination due to its transparent body and can be observed using more advanced techniques such as Selective Plane Illumination Microscopy or light sheet microscopy. *C. elegans'* transparency allows the entirety of organ development to be observed. The nematode exists as a hermaphrodite (XX), or occasionally as a male (Meneely et al., 2019). These hermaphrodites self-fertilize and can produce approximately 300 progeny when each animal reproduces (Meneely et al., 2019). The lifecycle of *C. elegans* is rapid: embryogenesis occurs in 12 h, larvae reach adulthood in 2.5 days, and their total lifespan is about 2 to 3 weeks. *C. elegans* also bears the distinction of being the first multicellular organism to have its whole genome sequenced (Consortium, 1998), and the first to have its connectome completed, meaning the complete structural connectivity of the worm's nervous system has been described (Cook et al., 2019). The worm's genome contains 97 million base pairs and approximately 20,000 genes, about 40% of which are analogous to genes found in humans (Consortium, 1998). *C. elegans* have 302 neurons and 56 glia, allowing *C. elegans* to react to changes in lighting, touch, temperature, salinity, and chemical gradients (Iliff and Xu, 2020). *C. elegans* uses temperature, oxygen, and salt-sensing neurons to detect and avoid CO_2. As an animal reliant on diffusion for gas exchange, the nematode avoids toxic levels of CO_2 by shunning environments where the gas exceeds 0.5% (Bretscher et al., 2011).

3.2. PM research

C. elegans has been used to assess the toxicity of multiple sources of PM including diesel exhaust, coal combustion, urban and rural ambient air, and from braking systems of cars (Sun et al., 2015; Wu et al., 2017; Wang et al., 2019; Volta et al., 2020). These include fine and ultrafine total particulate matter as well as PM extracts (Wang et al., 2019; Zhao et al., 2019; Haghani et al., 2019; Green and Kikis, 2020), and $PM_{2.5}$ (Sun et al., 2015; Yang et al., 2016; Wu et al., 2017). The methods of preparation of the PM samples were relatively similar to how they were prepared in the zebrafish studies. In *C. elegans* studies, PM was extracted using deionized water and sonication, or using dichloromethane as a solvent. Extracted PM solutions were then solvent exchanged and resuspended into ultrapure/deionized water, DMSO, or K buffer. *C. elegans* have been exposed to PM in a variety of plates, including glass petri dishes and welled plates of various sizes (24 to 96-well). Exposure concentrations of total PM mass in *C. elegans* studies varied but were generally 0.1–1200 mg/L (Volta et al., 2020; Zhao et al., 2019).

C. elegans have been used to assess the impacts of various PMs on lifespan and development and to identify potential mediating mechanisms. In Zhao et al. (2019), extractable organic material from $PM_{2.5}$ collected from air samplers on a university campus was found to induce oxidative stress, enhance metabolic enzyme activity, and shorten lifespan. The PM concentrations ranged from 0.1 to 100 mg/L, and significant responses were found at 10 and 100 mg/L, the second highest and highest concentrations. It was also found that exposure to antioxidants could rescue the lifespan attenuation due to $PM_{2.5}$ exposure. Coal combustion $PM_{2.5}$ was found to induce deficits in lifespan, development, reproduction, and locomotion behavior of *C. elegans* through changes in the expression patterns of genes related to the control of oxidative stress (Sun et al., 2015). Another study, Wu et al. (2017), found that exposure

to coal combustion-related fine particulate $PM_{2.5}$ dysregulated 25 microRNAs, including the mir-231-SMK-1-SOD-3/SOD-4/CTL-3 signaling pathway, many of which may play a critical role in oxidative stress. Furthermore, $PM_{2.5}$ collected during the Spring Festival in urban Beijing that was rich in heavy metals (i.e., Cd, Pb, Zn, and Cu) increased production of intestinal ROS and metallothioneins, proteins involved in the control of stress responses to heavy metals in *C. elegans* (Yang et al., 2016). These findings implicating oxidative stress are consistent with many studies in human bronchial epithelial cells. For example, one study found that ambient $PM_{2.5}$ induced oxidative stress and pro-inflammatory responses (Chung et al., 2020; Yuan et al., 2019). Moreover, Yan et al. (2021) assessed the toxicity of diesel exhaust PM in both *C. elegans* and human bronchial epithelial cells in the same study and found that exposures of 1 and 10 μg/mL DEP resulted in significantly impaired locomotion and dopaminergic disorders, and increased expression of heat shock protein-16.2, a protein involved in cellular stress responses (Yan et al., 2021). Human bronchial epithelial cells exposed to the same sample had decreased viability, increased production of reactive oxygen species (ROS), DNA damage, and increased cell death per increase in dose. Chung et al. (2020) also found that exposure to traffic-related $PM_{2.5}$ at a range of approximately 1 to 1600 mg/ml significantly decreased *C. elegans* lifespan, locomotor behaviors, and brood size in a dose-dependent fashion (Chung et al., 2020). Supporting that, Wang et al. (2019) studied the transgenerational effects of DEP exposure and found that DEP exposure impaired brood size in the F2 to F5 generations and identified signaling pathways involved in DEP-induced apoptosis (Wang et al., 2019). DEP has also been linked to apoptosis and autophagy in human bronchial epithelial cells (Colasanti et al., 2018). Table 4 is a summary of previous PM studies involving *C. elegans*.

3.3. Limitations

The combination of the short, invariant lifespan, ease of assays, ample genetic, molecular, and genomic tools, and evolutionary conservation has allowed *C. elegans* to develop into a popular model system for research. *C. elegans* does suffer from drawbacks as a model organism as it lacks many of the organ structures relevant to human health, making it challenging to extrapolate findings. Furthermore, *C. elegans* culture depends on a bacterial food source, traditionally *E. coli*, to grow in the laboratory. Past studies have found that differences in bacterial metabolism can alter the response of *C. elegans* to drug treatments (Garcia-Gonzalez et al., 2017; Scott et al., 2017), and therefore PM toxicity studies may have to consider the potential confounding effect of *C.elegans* food when presenting their findings. Attempts to grow nematodes in a chemically defined, axenic medium (a culture free from living organisms except for the organism of interest) have met roadblocks, but more recent work using liposome-based nanoparticle culturing shows promise (Flavel et al., 2018). It should also be noted that *C. elegans'* traditional methods of exposure, immersion or feeding, is a clear contrast and limitation relative to rodent studies involving inhalation exposure to PM, the more relevant route of exposure (Lee et al., 2021). Furthermore, *C. elegans* are far more primitive, lacking the complex mammalian respiratory system found in rodents. Despite these limitations, responses to chemical toxicants in nematodes significantly overlap with zebrafish developmental screens (Boyd et al., 2016).

4. *Drosophila melanogaster*

4.1. Characteristics

Drosophila melanogaster is a species of fly that has been used as a research model since the early 1900's (Morgan, 1910). *Drosophila* is commonly known as the "fruit fly", owing to its tendency to lay its eggs in fermenting fruit. The fly is yellow–brown colored with red eyes and transverse black rings on its abdomen. *Drosophila* exhibit sexual dimorphism with females averaging about 2.5 mm in size, while the males are slightly smaller and have a distinct black patch on their abdomen. The fruit fly is popular for its rapid life cycle, large number of offspring per generation, and well understood genetics (Greenspan, 2004). In optimal growth conditions (25 °C), *D. melanogaster*'s lifespan is about 50 days from egg to death. Females lay about 400 eggs per clutch into fermenting fruit or other suitable material. There are four stages in the life cycle of *Drosophila*: embryo, larva, pupa, adult. The development time from egg to adult in ideal conditions is 8.5 days (Calap-Quintana et al., 2017; Ong et al., 2015; Pandey and Nichols, 2011; Reiter et al., 2001). The flies used in these studies typically followed a standard *Drosophila* corn-sucrose yeast medium diet and were reared at around 22 °C with a 12:12 h light/dark cycle. In addition, the *Drosophila* genome is 60% homologous to humans and about 77% of the genes responsible for causing diseases in humans have homologues in flies (Reiter et al., 2001). *Drosophila* has only four pairs of chromosomes – an X/Y pair and three autosomes known as 2, 3, and 4. The genome of *Drosophila* was sequenced in 2000 and is curated at the Flybase Database (Adams et al., 2000). The genome consists of 139.5 million base pairs and contains around 15,682 genes. Sex in *Drosophila* is determined by the X:A ratio of X chromosomes to autosomes. *Drosophila* breathe using spiracles protected by hydrophobic hairs (Samakovlis et al., 1996). These spiracles lead to the fly's tracheal system, a branched network of epithelial tubes responsible in larva for the longitudinal transport of oxygen through passive diffusion (Krogh, 1920).

Drosophila are also excellent models for developmental studies. For example, developmental studies on cell fate determination, neuronal development, and organogenesis after exposure to ambient $PM_{2.5}$ can be carried out in the embryonic stage, while the final stage of larval development, the third instar larva, can be used for developmental, physiological, and behavioral studies (Ruben et al., 2018). Another benefit of using *Drosophila* as a model is the anatomical similarity of structures like the brain, heart, lung, kidney, liver, and gut to those of humans (Pandey and Nichols, 2011). For example, the *Drosophila* fat body functions similarly to the human liver. The fat body uses lipoprotein particles to transport lipids to peripheral tissues, while lipid metabolism in *Drosophila* is regulated by insulin signaling (Canavoso et al., 2001; DiAngelo and Birnbaum, 2009).

4.2. PM research

Drosophila has been used to assess the toxicity of multiple sources of PM including tobacco smoke, coal combustion byproducts, and rural/urban ambient air samples (Sadiq and Altaany 2014; Alija et al., 2016), de Santana et al., 2018, Ruben et al., 2018; Pandey et al., 2020; Thimmegowda et al., 2020). Like in the other models, *Drosophila* studies assessed the impacts of whole PM, organic extracts, and water-soluble extracts (Sadiq and Altaany 2014; Wang et al., 2017b; Pandey et al., 2020). The PM exposure period included repeated short exposures of 30 min per-day to cigarette-derived PM aerosol (Ruben et al., 2018), injection of cigarette smoke filtrate at concentrations up to 2.5% smoke in saline in 2–3 day old flies (Sadiq and Altaany, 2014), or by feeding flies TPM and PM_{10} from urban air samples (A Delgado-Rodríguez et al., 1999).

A common endpoint of PM toxicity studies in *Drosophila* is a somatic mutation and recombination test (SMART) used to study PM_{10} and total suspended particles, which were sourced from urban environments, extracted in methanol and then dried and resuspended into 3% ethanol (A Delgado-Rodríguez et al., 1999; Dihl et al., 2008). This is also known as a wing-spot test and is used to evaluate genotoxicity. For example, A Delgado-Rodríguez et al. (1999) found that PM_{10} was more genotoxic than total suspended particulate in *Drosophila* larvae exposed to PM via their feed from 3 to 5 days old. This is consistent with Jarvis et al. (2018), which found that coarse PM and its organic extract elicits a genotoxic response in human alveolar epithelial cells. A more recent study (Wang et al., 2017), found that exposure of adult flies to

Table 5
PM Studies in *D. melanogaster*.

Study/Reference	PM type	Exposure method	Concentrations	Major findings
Sadiq and Altaany 2014	Cigarette smoke filtrate (SF)	Flies injected intraperitoneally	0.2 μL in 0.45% NaCl saline	Stage specific mutagenicity; sex-linked recessive lethal mutations (spermatocytes/early spermatogonia) and induced mosaic mutations (late spermatogonia)
de Santana et al., 2018	Rural-urban $PM_{2.5}$	Flies (n = 120) were exposed for 6 days per site	Ambient concentrations only	Genetic damage as per the COMET Assay in urban environments
Alija et al., 2016	Coal fly ash	Ingestion	Fly ash added to nutrient medium at concentrations of 1%, 2%, and 3%	Increased recessive mutations at higher ingested concentrations
Ruben et al., 2018	Cigarette smoke-derived PM	Chronic exposure	30-minute exposure regimen	COPD-like phenotype, altered TGF-β, Nrf2 and JAK/STAT signaling pathways; oltipraz increased survivability of exposed flies
Pandey et al., 2020	Candle soot-derived nanoparticles	Developmental exposure: larvae were raised on food laced with concentrations of candle soot-derived nanoparticles	50 μg/ml, 100 μg/ml, and 250 μg/ml	Exposure did not affect the developmental period of the larva, but it did diminish reproductive performance later in life. A moderate level of cytotoxicity was observed.
Thimmegowda et al., 2020	Rural-urban PM	Flies (n = 1000) were exposed for 7 days per site	Ambient concentrations only	Differences in survival, behavior, heart rate, blood cell count, and the expression of genes related to stress, immunity, and metabolism.
Wang et al., 2017b	Concentrated ambient $PM_{2.5}$	whole-body ambient inhalational exposure to aerosolized $PM_{2.5}$	3, 4, 52, 54, 73, and 80 μg/m^3	Exposure-induced premature mortality, activation of pro-inflammatory signaling pathways, significantly increased whole-body and circulating glucose levels
A Delgado-Rodríguez 1999	Ambient Pm_{10} and total suspended particles (TSP) from two locations in Mexico City	Chronic treatment of 48 hrs, ingestion	1 ml of Dehydrated sample per 1 ml of ethanol, added to feeding medium	SMART assay (wing spot test) found higher genotoxic activity at Merced, and that the PM_{10} was more genotoxic than TSP
Dihl et al., 2008	Ambient Pm_{10} and TSP from Canoas, Brazil in the spring and summer	*Drosophila* eggs were grown in vials containing medium rehydrated with 5 ml of test or control solutions	PM_{10}: 28–32 μg/m3 TSP: 42–89 μg/m3	SMART assay (wing spot test) found PM10 genotoxic activity induced mitotic recombination; TSP induced recombination and point mutation

Studies listed in order in which they appear in manuscript.

concentrations on average of 80 μg/m^3 of urban $PM_{2.5}$ via an exposure chamber leads to premature mortality through induction of inflammation, oxidative stress, and metabolic abnormality in *Drosophila*. Specifically, they found that exposure to $PM_{2.5}$ increased glucose levels and expression of Ilp2 and Ilp5 (insulin-like peptide 2&5, respectively), indicating $PM_{2.5}$ exposure is linked to insulin dysregulation in *Drosophila*. Interestingly, higher $PM_{2.5}$ exposure has been found to induce insulin resistance in humans according to Li et al. (2017) and that elevated $PM_{2.5}$ levels were associated with higher prevalence of type 2 diabetes (Liu et al., 2019). Genotoxicity from exposures to rural and urban PM were studied in fly larvae on site and through a Comet assay of *Drosophila* hemolymph (analogous to blood in vertebrates) cells exposed to ambient PM by de Santana et al. (2018), who found that urban PM significantly degraded *Drosophila* hemolymph DNA compared to rural and filtered air exposures, implicating metals (e.g, aluminum, silicon, sulfur, potassium, calcium, titanium, and iron) found in the urban samples. Injected cigarette smoke filtrate was also found to cause mutations during spermatogenesis of 2 to 3-day-old wild-type *Drosophila* by Sadiq and Altaany (2014), indicating that cigarette smoke may have greater genotoxicity than previously predicted. Researchers found that exposure to ambient cigarette smoke, which is rich in PM, caused higher resting metabolic rates and premature death in adult *Drosophila* (Ruben et al., 2018). Interestingly, like in other models after PM exposure, transcriptomic analysis focusing on oxidative stress signaling pathways in this study found that the Nrf2 signaling pathway was altered after exposure to cigarette smoke PM (Ruben et al., 2018). Table 5 is a summary of previous PM studies involving *Drosophila*.

4.3. Limitations

There are, however, some drawbacks in the use of *Drosophila*. Questions of equivalent dose arise with each exposure method selected for the model. For instance, flies can be exposed to PM through feeding, ambient air inhalation, or injection. Injection of PM allows researchers to monitor the exact amount the flies are exposed to, but aerosol

Table 6
Advantages and Disadvantages of Each Model.

	Advantages	Disadvantages
D. rerio	Transgenerational studies possible. Highly conserved biological mechanisms. Behavioral assays possible. High fecundity. Optical transparency of early stages. Complete genome sequence. Strong genetic tools available. Very rapid development. Nearly all mammalian genes have a zebrafish counterpart.	Mode of exposure different from humans. Requires an aquatic facility. Exothermic (cold-blooded) . Chorion present up to 48 hpf: interference with drug diffusion. Genome duplication. Anatomical differences with mammals. Zebrafish embryos cannot be stored frozen, necessitating transfers of live adults to maintain stocks.
C. elegans	Ease of growth. Rapid development and reproduction. Short lifespan. Complete genome sequence. Strong genetic tools available. Self-fertilizing hermaphrodite. Can be cryogenically frozen.	Mode of exposure different from humans. Small size. Lower invertebrate: many physiological functions are not conserved.
D. melanogaster	Easy and cheap to maintain in large quantities. Short generation time. Fully sequenced and annotated genome. Good conservation of cellular process and signaling pathways. Mode of exposure similar to humans	Lower order organism; extrapolation to vertebrates more difficult. Difficult to measure behavior. *Drosophila* embryos cannot be stored frozen, necessitating transfers of live adults to maintain stocks. Immune system not as complex and adaptive as in vertebrates.

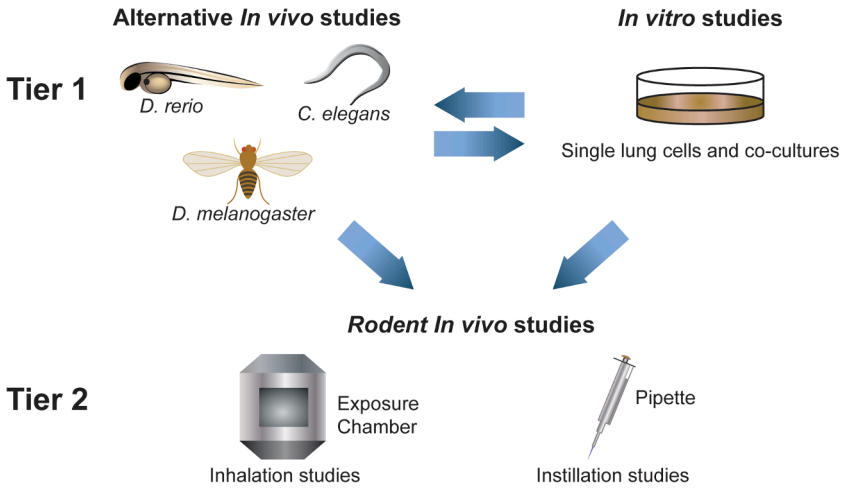

Fig. 2. Tiered approach for screening the toxicity of PM sources. Alternative *in vivo* studies can complement *in vitro* cell culture studies as first tier assays to inform targeted assessments of toxicity in higher order mammalian models in tier two.

exposure, like in rodent models, better mimics natural routes of human exposure. However, aerosol exposures in *Drosophila* are limited by the lack of accurate dosimetry. Particle deposition of PM after "inhalation" has, to our knowledge, not been studied in Drosophila and as such the extent to which it compares with mammalian models is unknown. Despite the drawbacks, the commonality in exposure route and organ structure makes responses in *Drosophila* translatable to human health. *Drosophila* may also have utility as a model for studying the ecotoxicological effect of particulate matter – as evidenced by the findings of Thimmegowda et al. (2020), wherein it was discovered that *Drosophila* had similar molecular and physiological differences to PM as the Giant Asian honeybee, *Apis dorsata*.

5. Outlook

In conclusion, alternative *in vivo* models have well understood genetics, a foundation of decades of research, and can help bridge the gap between *in vitro* and mammalian *in vivo* studies. Of the three models, *Drosophila* is the only model that "inhales" from ambient air with its spiracles, more closely resembling human exposure. Consequently, *Drosophila* can be exposed to a PM aerosol, circumventing the chemistry, extraction, and agglomeration challenges with PM in media. *C. elegans* and larval zebrafish have the benefit of having translucent skin, which facilitates measures of function and morphology of internal organs with microscopy and imaging techniques. Zebrafish and *Drosophila* offer the advantages of having more advanced mammalian-like organs, allowing for extrapolation of effects at the systems level. The advantages and disadvantages of the alternative *in vivo* models in the context of PM research has been summarized in Table 6.

Together, these models can be imagined as one set of toxicological tools in a tiered approach (Fig. 2) that includes assessment of responses in human lung epithelial cells and mammalian *in vivo* models (i.e., rodents). Alternative *in vivo* model assessments may be useful as first tier assays for screening toxicity of multiple PM sources as well as providing insight into mechanisms of action and development of adverse outcome pathways (Villeneuve et al., 2014). In such a high throughput context, researchers may explore development of a standard battery of high throughput assays that measure a combination of endpoints that may include transcriptomics, behavior, and/or phenotyping among others. Higher throughput alternative model approaches may also be coupled with bioassay-directed fractionation to identify potent chemical classes in PM (DeMarini et al., 2004). Alternative model data may also feed into more targeted assessments in higher order *in vivo* models that are carried out to investigate the toxicity of more toxic PM sources in greater depth. Alternative model assessments may also be performed secondary to initial assessments in lung epithelial cell lines to gain information regarding systemic responses or more complex processes like inflammation or altered function. For example, one study showed that heart rate responses in zebrafish were predictive of myocardial membrane activity in humans Milan et al. (2003), an important finding in this context given that PM causes adverse cardiovascular responses in people (Brook et al., 2010). Moreover, elegant neurobehavioral assays have been developed in zebrafish (Jarema et al., 2015) and *Drosophila* (Haddadi et al., 2016) that can be used to understand the growing link between PM exposure and adverse neurobehavioral outcomes in humans (Sram et al., 2017). Taken together, their homology in biological responsiveness and the greater throughput they afford suggest that these models may facilitate toxicity characterization of PM from multiple sources and aid in the identification of the most toxic PM components.

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Jacob Smoot: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Stephanie Padilla:** Conceptualization, Writing – review & editing. **Aimen K. Farraj:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

A Delgado-Rodríguez, R. O.-M., R Villalobos-Pietrini, S Gómez-Arroyo, U Graf. (1999). Genotoxicity of organic extracts of airborne particles in somatic cells of Drosophila melanogaster. *Chemosphere, 39*, 33-43. doi:10.1016/s0045-6535(98)00586-4.

Adams, M.D., Celniker, S.E., Holt, R.A., Evans, C.A., Gocayne, J.D., Amanatides, P.G., et al., 2000. The genome sequence of Drosophila melanogaster. Science 287 (5461), 2185–2195. https://doi.org/10.1126/science.287.5461.2185.

Alija, A.J., Bajraktari, I.D., Muharremi, H., Bresgen, N., Eckl, P.M., 2016. Effects of pollutants from power plants in Kosova on genetic loads of Drosophila melanogaster. Toxicol. Ind. Health 32 (7), 1310–1317. https://doi.org/10.1177/0748233714558083.

Babić, S., Čižmek, L., Maršavelski, A., Malev, O., Pflieger, M., Strunjak-Perović, I., Popović, N.T., Čož-Rakovac, R., Trebše, P., 2021. Utilization of the zebrafish model to unravel the harmful effects of biomass burning during Amazonian wildfires. Sci. Rep. 11 (1) https://doi.org/10.1038/s41598-021-81789-1.

Bai, K.J., Chuang, K.J., Wu, S.M., Chang, L.T., Chang, T.Y., Ho, K.F., Chuang, H.C., 2018. Effects of diesel exhaust particles on the expression of tau and autophagy proteins in human neuroblastoma cells. Environ. Toxicol. Pharmacol. 62, 54–59. https://doi.org/10.1016/j.etap.2018.06.007.

Bai, N., van Eeden, S.F., 2013. Systemic and vascular effects of circulating diesel exhaust particulate matter. Inhal Toxicol 25 (13), 725–734. https://doi.org/10.3109/08958378.2013.844749.

Barnhill, L.M., Khuansuwan, S., Juarez, D., Murata, H., Araujo, J.A., Bronstein, J.M., 2020. Diesel exhaust extract exposure induces neuronal toxicity by disrupting autophagy. Toxicol. Sci. 176 (1), 193–202. https://doi.org/10.1093/toxsci/kfaa055.

Bertelli, P.R., Biegelmeyer, R., Rico, E.P., Klein-Junior, L.C., Toson, N.S.B., Minetto, L., Henriques, A.T., 2017. Toxicological profile and acetylcholinesterase inhibitory potential of Palicourea deflexa, a source of beta-carboline alkaloids. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 201, 44–50. https://doi.org/10.1016/j.cbpc.2017.09.003.

Boyd, W.A., Smith, M.V., Co, C.A., Pirone, J.R., Rice, J.R., Shockley, K.R., Freedman, J. H., 2016. Developmental effects of the ToxCast Phase I and Phase II chemicals in Caenorhabditis elegans and corresponding responses in zebrafish, rats, and rabbits. Environ. Health Perspect. 124 (5), 586–593. https://doi.org/10.1289/ehp.1409645.

Bretscher, A.J., Kodama-Namba, E., Busch, K.E., Murphy, R.J., Soltesz, Z., Laurent, P., de Bono, M., 2011. Temperature, oxygen, and salt-sensing neurons in C. elegans are carbon dioxide sensors that control avoidance behavior. Neuron 69 (6), 1099–1113. https://doi.org/10.1016/j.neuron.2011.02.023.

Brook, R.D., Rajagopalan, S., Pope, C.A., Brook, J.R., Bhatnagar, A., Diez-Roux, A.V., Holguin, F., Hong, Y., Luepker, R.V., Mittleman, M.A., Peters, A., Siscovick, D., Smith, S.C., Whitsel, L., Kaufman, J.D., 2010. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 121 (21), 2331–2378. https://doi.org/10.1161/CIR.0b013e3181dbece1.

Caballero-Gallardo, K., Wirbisky-Hershberger, S.E., Olivero-Verbel, J., de la Rosa, J., Freeman, J.L., 2018. Embryonic exposure to an aqueous coal dust extract results in gene expression alterations associated with the development and function of connective tissue and the hematological system, immunological and inflammatory disease, and cancer in zebrafish. Metallomics 10 (3), 463–473. https://doi.org/10.1039/c7mt00300e.

Calap-Quintana, P., Gonzalez-Fernandez, J., Sebastia-Ortega, N., Llorens, J.V., Molto, M. D., 2017. Drosophila melanogaster models of metal-related human diseases and metal toxicity. Int. J. Mol. Sci. 18 (7) https://doi.org/10.3390/ijms18071456.

Canavoso, L.E., Jouni, Z.E., Karnas, K.J., Pennington, J.E., Wells, M.A., 2001. Fat metabolism in insects. Annu. Rev. Nutr. 21, 23–46. https://doi.org/10.1146/annurev.nutr.21.1.23.

Cassar, S., Adatto, I., Freeman, J.L., Gamse, J.T., Iturria, I., Lawrence, C., Zon, L.I., 2020. Use of Zebrafish in Drug Discovery Toxicology. Chem. Res. Toxicol. 33 (1), 95–118. https://doi.org/10.1021/acs.chemrestox.9b00335.

Cen, J., Jia, Z. L., Zhu, C. Y., Wang, X. F., Zhang, F., Chen, W. Y., et al. (2020). Particulate matter (PM10) induces cardiovascular developmental toxicity in zebrafish embryos and larvae via the ERS, Nrf2 and Wnt pathways. *Chemosphere, 250*, 126288. doi: 10.1016/j.chemosphere.2020.126288.

Chung, M.-C., Huang, K.-L., Avelino, J.L., Tayo, L.L., Lin, C.-C., Tsai, M.-H., Lin, S.-L., Mansor, W.N.W., Su, C.-K., Huang, S.-T., 2020. Toxic assessment of heavily traffic-related fine particulate matter using an in-vivo wild-type Caenorhabditis elegans model. Aerosol Air Qual. Res. 20 (9), 1974–1986. https://doi.org/10.4209/aaqr.2020.05.0192.

Colasanti, T., Fiorito, S., Alessandri, C., Serafino, A., Andreola, F., Barbati, C., Morello, F., Alfè, M., Di Blasio, G., Gargiulo, V., Vomero, M., Conti, F., Valesini, G., 2018. Diesel exhaust particles induce autophagy and citrullination in Normal Human Bronchial Epithelial cells. Cell Death Dis. 9 (11) https://doi.org/10.1038/s41419-018-1111-y.

Consortium C. e. S, 1998. Genome sequence of the nematode C. elegans: a platform for investigating biology. Science 282 (5396), 2012–2018. https://doi.org/10.1126/science.282.5396.2012.

Cook, S.J., Jarrell, T.A., Brittin, C.A., Wang, Y.i., Bloniarz, A.E., Yakovlev, M.A., Nguyen, K.C.Q., Tang, L.-H., Bayer, E.A., Duerr, J.S., Bülow, H.E., Hobert, O., Hall, D.H., Emmons, S.W., 2019. Whole-animal connectomes of both Caenorhabditis elegans sexes. Nature 571 (7763), 63–71. https://doi.org/10.1038/s41586-019-1352-7.

Dai, Y.L., Jiang, Y.F., Lu, Y.A., Yu, J.B., Kang, M.C., Jeon, Y.J., 2021. Fucoxanthin-rich fraction from Sargassum fusiformis alleviates particulate matter-induced inflammation in vitro and in vivo. Toxicol. Rep. 8, 349–358. https://doi.org/10.1016/j.toxrep.2021.02.005.

de Santana, S.L., Verçosa, C.J., de Araújo Castro, Í.F., de Amorim, É.M., da Silva, A.S., da Rocha Bastos, T.M., da Silva Neto, L.J., dos Santos, T.O., De França, E.J., Rohde, C., 2018. Drosophila melanogaster as model organism for monitoring and analyzing genotoxicity associated with city air pollution. Environ. Sci. Pollut. Res. Int. 25 (32), 32409–32417. https://doi.org/10.1007/s11356-018-3186-5.

DeMarini, D.M., Brooks, L.R., Warren, S.H., Kobayashi, T., Gilmour, M.I., Singh, P., 2004. Bioassay-directed fractionation and salmonella mutagenicity of automobile and forklift diesel exhaust particles. Environ. Health Perspect. 112 (8), 814–819. https://doi.org/10.1289/ehp.6578.

DiAngelo, J.R., Birnbaum, M.J., 2009. Regulation of fat cell mass by insulin in Drosophila melanogaster. Mol. Cell. Biol. 29 (24), 6341–6352. https://doi.org/10.1128/MCB.00675-09.

Dihl, R.R., da Silva, C.G.A., Amaral, V.S.d., Reguly, M.L., de Andrade, H.H.R., 2008. Mutagenic and recombinagenic activity of airborne particulates, PM10 and TSP, organic extracts in the Drosophila wing-spot test. Environ. Pollut. 151 (1), 47–52. https://doi.org/10.1016/j.envpol.2007.03.008.

Duan, J., Hu, H., Zhang, Y., Feng, L., Shi, Y., Miller, M.R., Sun, Z., 2017. Multi-organ toxicity induced by fine particulate matter PM2.5 in zebrafish (Danio rerio) model. Chemosphere 180, 24–32. https://doi.org/10.1016/j.chemosphere.2017.04.013.

Fann, N., Lamson, A.D., Anenberg, S.C., Wesson, K., Risley, D., Hubbell, B.J., 2012. Estimating the national public health burden associated with exposure to ambient PM2.5 and ozone. Risk Anal. 32 (1), 81–95. https://doi.org/10.1111/j.1539-6924.2011.01630.x.

Flavel, M. R., Mechler, A., Shahmiri, M., Mathews, E. R., Franks, A. E., Chen, W., et al. (2018). Growth of Caenorhabditis elegans in defined media is dependent on presence of particulate matter. *G3 (Bethesda), 8*(2), 567-575. doi:10.1534/g3.117.300325.

Freires, I.A., Sardi, J.C., de Castro, R.D., Rosalen, P.L., 2017. Alternative animal and non-animal models for drug discovery and development: Bonus or burden? Pharm. Res. 34 (4), 681–686. https://doi.org/10.1007/s11095-016-2069-z.

Garcia-Gonzalez, A.P., Ritter, A.D., Shrestha, S., Andersen, E.C., Yilmaz, L.S., Walhout, A. J.M., 2017. Bacterial metabolism affects the C. elegans response to cancer chemotherapeutics. Cell 169 (3), 431–441 e438. https://doi.org/10.1016/j.cell.2017.03.046.

Goessling, W., Sadler, K.C., 2015. Zebrafish: an important tool for liver disease research. Gastroenterology 149 (6), 1361–1377. https://doi.org/10.1053/j.gastro.2015.08.034.

Gómez-Orte, E., Cornes, E., Zheleva, A., Sáenz-Narciso, B., de Toro, M., Iñiguez, M., López, R., San-Juan, J.-F., Ezcurra, B., Sacristán, B., Sánchez-Blanco, A., Cerón, J., Cabello, J., 2018. Effect of the diet type and temperature on the C. elegans transcriptome. Oncotarget 9 (11), 9556–9571.

Green, E. H., & Kikis, E. A. (2020). Determining the effects of nanoparticulate air pollution on proteostasis in Caenorhabditis elegans. *PLoS One, 15*(12), e0243419. doi:10.1371/journal.pone.0243419.

Greenspan, R., 2004. Fly Pushing: The Theory and Practice of Drosophila genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor (NY).

Gross, A.D., Tabanca, N., Islam, R., Ali, A., Khan, I.A., Kaplancikli, Z.A., Bloomquist, J.R., 2017. Toxicity and synergistic activities of Chalcones Against Aedes aegypti (Diptera: Culicidae) and Drosophila melanogaster (Diptera: Drosophilidae). J. Med. Entomol. 54 (2), 382–386. https://doi.org/10.1093/jme/tjw183.

Haddadi, M., Jahromi, S.R., Nongthomba, U., Shivanandappa, T., Ramesh, S.R., 2016. 4-Hydroxyisophthalic acid from Decalepis hamiltonii rescues the neurobehavioral deficit in transgenic Drosophila model of taupathies. Neurochem. Int. 100, 78–90. https://doi.org/10.1016/j.neuint.2016.09.007.

Haghani, A., Dalton, H. M., Safi, N., Shirmohammadi, F., Sioutas, C., Morgan, T. E., et al. (2019). Air pollution alters Caenorhabditis elegans development and lifespan: responses to traffic-related nanoparticulate matter. *J Gerontol A Biol Sci Med Sci, 74*(8), 1189-1197. doi:10.1093/gerona/glz063.

Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., Muffato, M., Collins, J.E., Humphray, S., McLaren, K., Matthews, L., McLaren, S., Sealy, I., Caccamo, M., Churcher, C., Scott, C., Barrett, J.C., Koch, R., Rauch, G.-J., White, S., Chow, W., Kilian, B., Quintais, L.T., Guerra-Assunção, J.A., Zhou, Y.i., Gu, Y., Yen, J., Vogel, J.-H., Eyre, T., Redmond, S., Banerjee, R., Chi, J., Fu, B., Langley, E., Maguire, S.F., Laird, G.K., Lloyd, D., Kenyon, E., Donaldson, S., Sehra, H., Almeida-King, J., Loveland, J., Trevanion, S., Jones, M., Quail, M., Willey, D., Hunt, A., Burton, J., Sims, S., McLay, K., Plumb, B., Davis, J., Clee, C., Oliver, K., Clark, R., Riddle, C., Elliott, D., Threadgold, G., Harden, G., Ware, D., Begum, S., Mortimore, B., Kerry, G., Heath, P., Phillimore, B., Tracey, A., Corby, N., Dunn, M., Johnson, C., Wood, J., Clark, S., Pelan, S., Griffiths, G., Smith, M., Glithero, R., Howden, P., Barker, N., Lloyd, C., Stevens, C., Harley, J., Holt, K., Panagiotidis, G., Lovell, J., Beasley, H., Henderson, C., Gordon, D., Auger, K., Wright, D., Collins, J., Raisen, C., Dyer, L., Leung, K., Robertson, L., Ambridge, K., Leongamornlert, D., McGuire, S., Gilderthorp, R., Griffiths, C., Manthravadi, D., Nichol, S., Barker, G., Whitehead, S., Kay, M., Brown, J., Murnane, C., Gray, E., Humphries, M., Sycamore, N., Barker, D., Saunders, D., Wallis, J., Babbage, A., Hammond, S., Mashreghi-Mohammadi, M., Barr, L., Martin, S., Wray, P., Ellington, A., Matthews, N., Ellwood, M., Woodmansey, R., Clark, G., Cooper, J.D., Tromans, A., Grafham, D., Skuce, C., Pandian, R., Andrews, R., Harrison, E., Kimberley, A., Garnett, J., Fosker, N., Hall, R., Garner, P., Kelly, D., Bird, C., Palmer, S., Gehring, I., Berger, A., Dooley, C. M., Ersan-Ürün, Z., Eser, C., Geiger, H., Geisler, M., Karotki, L., Kirn, A., Konantz, J., Konantz, M., Oberländer, M., Rudolph-Geiger, S., Teucke, M., Lanz, C., Raddatz, G., Osoegawa, K., Zhu, B., Rapp, A., Widaa, S., Langford, C., Yang, F., Schuster, S.C., Carter, N.P., Harrow, J., Ning, Z., Herrero, J., Searle, S.M.J., Enright, A., Geisler, R., Plasterk, R.H.A., Lee, C., Westerfield, M., de Jong, P.J., Zon, L.I., Postlethwait, J.H., Nüsslein-Volhard, C., Hubbard, T.J.P., Crollius, H.R., Rogers, J., Stemple, D.L., 2013. The zebrafish reference genome sequence and its relationship to the human genome. Nature 496 (7446), 498–503. https://doi.org/10.1038/nature12111.

Hughes, M. C., Zimmer, A. M., Perry, S. F. (2019). Role of internal convection in respiratory gas transfer and aerobic metabolism in larval zebrafish (Danio rerio). *Am. J. Physiol. Regul. Integr. Comp. Physiol., 316*(3), R255-R264. doi:10.1152/ajpregu.00315.2018.

Iliff, A.J., Xu, X.Z.S., 2020. C. elegans: a sensible model for sensory biology. J. Neurogenet. 34 (3–4), 347–350. https://doi.org/10.1080/01677063.2020.1823386.

Jarema, K.A., Hunter, D.L., Shaffer, R.M., Behl, M., Padilla, S., 2015. Acute and developmental behavioral effects of flame retardants and related chemicals in

zebrafish. Neurotoxicol. Teratol. 52 (Pt B), 194–209. https://doi.org/10.1016/j.ntt.2015.08.010.
Jarvis, I.W.H., Enlo-Scott, Z., Nagy, E., Mudway, I.S., Tetley, T.D., Arlt, V.M., Phillips, D. H., 2018. Genotoxicity of fine and coarse fraction ambient particulate matter in immortalised normal (TT1) and cancer-derived (A549) alveolar epithelial cells. Environ. Mol. Mutagen. 59 (4), 290–301. https://doi.org/10.1002/em.22166.
Jiang, Y., Li, J., Ren, F., Ji, C., Aniagu, S., Chen, T., 2019. PM2.5-induced extensive DNA methylation changes in the heart of zebrafish embryos and the protective effect of folic acid. Environ. Pollut. 255 (Pt 3), 113331 https://doi.org/10.1016/j.envpol.2019.113331.
Johnson, N.M., Hoffmann, A.R., Behlen, J.C., Lau, C., Pendleton, D., Harvey, N., Shore, R., Li, Y., Chen, J., Tian, Y., Zhang, R., 2021. Air pollution and children's health-a review of adverse effects associated with prenatal exposure from fine to ultrafine particulate matter. Environ. Health Prev. Med. 26 (1) https://doi.org/10.1186/s12199-021-00995-5.
Kenneth D. Poss, L. G. W., Mark T. Keating*. (2002). Heart Regeneration in Zebrafish.
Kim, J. Y., Lee, E. Y., Choi, I., Kim, J., Cho, K. H. (2015). Effects of the Particulate Matter (2).(5) (PM(2).(5)) on lipoprotein metabolism, uptake and degradation, and embryo toxicity. *Mol Cells, 38*(12), 1096-1104. doi:10.14348/molcells.2015.0194.
Kim, Y.H., King, C., Krantz, T., Hargrove, M.M., George, I.J., McGee, J., Copeland, L., Hays, M.D., Landis, M.S., Higuchi, M., Gavett, S.H., Gilmour, M.I., 2019. The role of fuel type and combustion phase on the toxicity of biomass smoke following inhalation exposure in mice. Arch. Toxicol. 93 (6), 1501–1513. https://doi.org/10.1007/s00204-019-02450-5.
Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., Schilling, T.F., 1995. Stages of embryonic development of the zebrafish. Dev. Dyn. 203 (3), 253–310. https://doi.org/10.1002/aja.1002030302.
Kiyama, Y., Miyahara, K., Ohshima, Y., 2012. Active uptake of artificial particles in the nematode Caenorhabditis elegans. J. Exp. Biol. 215 (Pt 7), 1178–1183. https://doi.org/10.1242/jeb.067199.
Krogh, A. (1920). Studien fiber Tracheenrespiration.
Kuhnert, A., Vogs, C., Altenburger, R., Kuster, E., 2013. The internal concentration of organic substances in fish embryos–a toxicokinetic approach. Environ. Toxicol. Chem. 32 (8), 1819–1827. https://doi.org/10.1002/etc.2239.
Lee, S. H., Chen, Y. H., Chien, C. C., Yan, Y. H., Chen, H. C., Chuang, H. C., et al. (2021). Three month inhalation exposure to low-level PM2.5 induced brain toxicity in an Alzheimer's disease mouse model. *PLoS One, 16*(8), e0254587. doi:10.1371/journal.pone.0254587.
Leung, L.C., Wang, G.X., Madelaine, R., Skariah, G., Kawakami, K., Deisseroth, K., Urban, A.E., Mourrain, P., 2019. Neural signatures of sleep in zebrafish. Nature 571 (7764), 198–204.
Li, H., Cai, J., Chen, R., Zhao, Z., Ying, Z., Wang, L., Chen, J., Hao, K., Kinney, P.L., Chen, H., Kan, H., 2017. Particulate matter exposure and stress hormone levels: a randomized, double-blind, crossover trial of air purification. Circulation 136 (7), 618–627. https://doi.org/10.1161/CIRCULATIONAHA.116.026796.
Liu, F., Chen, G., Huo, W., Wang, C., Liu, S., Li, N., Mao, S., Hou, Y., Lu, Y., Xiang, H., 2019. Associations between long-term exposure to ambient air pollution and risk of type 2 diabetes mellitus: a systematic review and meta-analysis. Environ. Pollut. 252, 1235–1245. https://doi.org/10.1016/j.envpol.2019.06.033.
Ma, M., Kler, S., Pan, Y.A., 2019. Structural neural connectivity analysis in zebrafish with restricted anterograde transneuronal viral labeling and quantitative brain mapping. Front. Neural Circuits 13, 85. https://doi.org/10.3389/fncir.2019.00085.
Manjunatha, B., Deekshitha, B., Seo, E., Kim, J., Lee, S.J., 2021. Developmental toxicity induced by particulate matter (PM2.5) in zebrafish (Danio rerio) model. Aquat. Toxicol. 238, 105928 https://doi.org/10.1016/j.aquatox.2021.105928.
Martin, W.K., Padilla, S., Kim, Y.H., Hunter, D.L., Hays, M.D., DeMarini, D.M., Hazari, M. S., Gilmour, M.I., Farraj, A.K., 2021. Zebrafish irritant responses to wildland fire-related biomass smoke are influenced by fuel type, combustion phase, and byproduct chemistry. J. Toxicol. Environ. Health A 84 (16), 674–688.
Martin, W.K., Tennant, A.H., Conolly, R.B., Prince, K., Stevens, J.S., DeMarini, D.M., Martin, B.L., Thompson, L.C., Gilmour, M.I., Cascio, W.E., Hays, M.D., Hazari, M.S., Padilla, S., Farraj, A.K., 2019. High-throughput video processing of heart rate responses in multiple wild-type embryonic zebrafish per imaging field. Sci. Rep. 9 (1), 145. https://doi.org/10.1038/s41598-018-35949-5.
Massarsky, A., Bone, A.J., Dong, W., Hinton, D.E., Prasad, G.L., Di Giulio, R.T., 2016. AHR2 morpholino knockdown reduces the toxicity of total particulate matter to zebrafish embryos. Toxicol. Appl. Pharmacol. 309, 63–76. https://doi.org/10.1016/j.taap.2016.08.024.
McLeish, J.A., Chico, T.J., Taylor, H.B., Tucker, C., Donaldson, K., Brown, S.B., 2010. Skin exposure to micro- and nano-particles can cause haemostasis in zebrafish larvae. Thromb Haemost 103 (4), 797–807. https://doi.org/10.1160/TH09-06-0413.
Meneely, P.M., Dahlberg, C.L., Rose, J.K., 2019. Working with worms: Caenorhabditis elegansas a model organism. Curr. Protocols Essential Lab. Tech. 19 (1) https://doi.org/10.1002/cpet.35.
Mesquita, S.R., Dachs, J., van Drooge, B.L., Castro-Jiménez, J., Navarro-Martín, L., Barata, C., Vieira, N., Guimarães, L., Piña, B., 2016. Toxicity assessment of atmospheric particulate matter in the Mediterranean and Black Seas open waters. Sci. Total Environ. 545-546, 163–170. https://doi.org/10.1016/j.scitotenv.2015.12.055.
Mesquita, S.R., van Drooge, B.L., Dall'Osto, M., Grimalt, J.O., Barata, C., Vieira, N., Guimarães, L., Piña, B., 2017. Toxic potential of organic constituents of submicron particulate matter (PM1) in an urban road site (Barcelona). Environ. Sci. Pollut. Res. Int. 24 (18), 15406–15415. https://doi.org/10.1007/s11356-017-9201-4.
Milan, D.J., Peterson, T.A., Ruskin, J.N., Peterson, R.T., MacRae, C.A., 2003. Drugs that induce repolarization abnormalities cause bradycardia in zebrafish. Circulation 107 (10), 1355–1358. https://doi.org/10.1161/01.cir.0000061912.88753.87.
Moorman, S.J., 2001. Development of sensory systems in zebrafish (Danio rerio). ILAR J 42 (4), 292–298. https://doi.org/10.1093/ilar.42.4.292.
Morgan, T.H., 1910. Sex limited inheritance in Drosophilia. Science 32 (812), 120–122. https://doi.org/10.1126/science.32.812.120.
Morikane, D., Zang, L., Nishimura, N., 2020. Evaluation of the percutaneous absorption of drug molecules in zebrafish. Molecules 25 (17). https://doi.org/10.3390/molecules25173974.
Nasiadka, A., Clark, M.D., 2012. Zebrafish breeding in the laboratory environment. ILAR J 53 (2), 161–168. https://doi.org/10.1093/ilar.53.2.161.
Ogura, Y., Kaneko, R., Ujibe, K., Wakamatsu, Y., Hirata, H., 2021. Loss of alphaklotho causes reduced motor ability and short lifespan in zebrafish. Sci. Rep. 11 (1), 15090. https://doi.org/10.1038/s41598-021-93909-y.
Oh, S.M., Kim, H.R., Park, Y.J., Lee, S.Y., Chung, K.H., 2011. Organic extracts of urban air pollution particulate matter (PM2.5)-induced genotoxicity and oxidative stress in human lung bronchial epithelial cells (BEAS-2B cells). Mutation Res.-Genetic Toxicol. Environ. Mutagenesis 723 (2), 142–151. https://doi.org/10.1016/j.mrgentox.2011.04.003.
Ong, C., Yung, L.Y., Cai, Y., Bay, B.H., Baeg, G.H., 2015. Drosophila melanogaster as a model organism to study nanotoxicity. Nanotoxicology 9 (3), 396–403. https://doi.org/10.3109/17435390.2014.940405.
Pandey, H., Saini, S., Singh, S.P., Gautam, N.K., Singh, S., 2020. Candle soot derived carbon nanoparticles: an assessment of cellular and progressive toxicity using Drosophila melanogaster model. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 228, 108646 https://doi.org/10.1016/j.cbpc.2019.108646.
Pandey, U.B., Nichols, C.D., 2011. Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. Pharmacol. Rev. 63 (2), 411–436. https://doi.org/10.1124/pr.110.003293.
Pope 3rd, C.A., Burnett, R.T., Thurston, G.D., Thun, M.J., Calle, E.E., Krewski, D., Godleski, J.J., 2004. Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease. Circulation 109 (1), 71–77. https://doi.org/10.1161/01.CIR.0000108927.80044.7F.
Porazzi, P., Calebiro, D., Benato, F., Tiso, N., Persani, L., 2009. Thyroid gland development and function in the zebrafish model. Mol. Cell. Endocrinol. 312 (1–2), 14–23. https://doi.org/10.1016/j.mce.2009.05.011.
Progatzky, F., Cook, H. T., Lamb, J. R., Bugeon, L., allman, M. J. (2016). Mucosal inflammation at the respiratory interface: a zebrafish model. *Am. J. Physiol. Lung Cell Mol. Physiol., 310*(6), L551-561. doi:10.1152/ajplung.00323.2015.
Reiter, L.T., Potocki, L., Chien, S., Gribskov, M., Bier, E., 2001. A systematic analysis of human disease-associated gene sequences in Drosophila melanogaster. Genome Res. 11 (6), 1114–1125. https://doi.org/10.1101/gr.169101.
Ren, F., Huang, Y., Tao, Y., Ji, C., Aniagu, S., Jiang, Y., Chen, T., 2020. Resveratrol protects against PM2.5-induced heart defects in zebrafish embryos as an antioxidant rather than as an AHR antagonist. Toxicol. Appl. Pharmacol. 398, 115029 https://doi.org/10.1016/j.taap.2020.115029.
Roper, C., Delgado, L.S., Barrett, D., Massey Simonich, S.L., Tanguay, R.L., 2019. PM2.5 filter extraction methods: implications for chemical and toxicological analyses. Environ. Sci. Technol. 53 (1), 434–442. https://doi.org/10.1021/acs.est.8b04308.
Ruben, P., Thiedmann, M., Bhandari, A., Mishra, N., Sinha, A., Häsler, R., et al. (2018). A Drosophila model of cigarette smoke induced COPD identifies Nrf2 signaling as an expedient target for intervention. *Aging, 10*, 2122-2135. doi:10.18632/aging.101536.
Rudich, P., Snoznik, C., Puleo, N., Lamitina, T., 2020. Measuring RAN Peptide Toxicity in C. elegans. J Vis Exp 158. https://doi.org/10.3791/61024.
Sadiq, M.F., Altaany, Z.N., 2014. Induction of complete and mosaic sex-linked recessive lethal mutations by cigarette smoke filtrate in Drosophila melanogaster. Drug Chem. Toxicol. 37 (2), 163–168. https://doi.org/10.3109/01480545.2013.834355.
Samakovlis, C., Hacohen, N., Manning, G., Sutherland, D.C., Guillemin, K., Krasnow, M. A., 1996. Development of the Drosophila tracheal system occurs by a series of morphologically distinct but genetically coupled branching events. Development. 122 (5), 1395–1407.
Santos, D., Luzio, A., Coimbra, A.M., 2017. Zebrafish sex differentiation and gonad development: a review on the impact of environmental factors. Aquat. Toxicol. 191, 141–163. https://doi.org/10.1016/j.aquatox.2017.08.005.
Scott, T.A., Quintaneiro, L.M., Norvaisas, P., Lui, P.P., Wilson, M.P., Leung, K.Y., Cabreiro, F., 2017. Host-microbe co-metabolism dictates cancer drug efficacy in C. elegans. Cell 169 (3), 442–456 e418. https://doi.org/10.1016/j.cell.2017.03.040.
Singleman, C., Holtzman, N.G., 2014. Growth and maturation in the zebrafish, Danio rerio: a staging tool for teaching and research. Zebrafish 11 (4), 396–406. https://doi.org/10.1089/zeb.2014.0976.
Song, Z., Zhang, Y., Zhang, H., Rajendran, R. S., Wang, R., Hsiao, C. D., . . . Liu, K. (2020). Isoliquiritigenin triggers developmental toxicity and oxidative stress-mediated apoptosis in zebrafish embryos/larvae via Nrf2-HO1/JNK-ERK/mitochondrion pathway. *Chemosphere, 246*, 125727. doi:10.1016/j.chemosphere.2019.125727.
Sram, R.J., Veleminsky Jr., M., Veleminsky Sr., M., Stejskalova, J., 2017. The impact of air pollution to central nervous system in children and adults. Retrieved from Neuro Endocrinol. Lett. 38 (6), 389–396. https://www.ncbi.nlm.nih.gov/pubmed/29298278.
Stednitz, S.J., Washbourne, P., 2020. Rapid progressive social development of zebrafish. Zebrafish 17 (1), 11–17. https://doi.org/10.1089/zeb.2019.1815.
Stevens, J. S., Padilla, S., DeMarini, D. M., Hunter, D. L., Martin, W. K., Thompson, L. C., et al. (2018). Zebrafish locomotor responses reveal irritant effects of fine particulate

matter extracts and a role for TRPA1. *Toxicol. Sci., 161*(2), 290-299. doi:10.1093/toxsci/kfx217.

Sun, L., Lin, Z., Liao, K., Xi, Z., Wang, D., 2015. Adverse effects of coal combustion related fine particulate matter (PM2.5) on nematode Caenorhabditis elegans. Sci. Total Environ. 512–513, 251–260. https://doi.org/10.1016/j.scitotenv.2015.01.058.

Takahashi, N., Mori, Y., 2011. TRP Channels as Sensors and Signal Integrators of Redox Status Changes. Front Pharmacol. 2, 58. https://doi.org/10.3389/fphar.2011.00058.

Thimmegowda, G.G., Mullen, S., Sottilare, K., Sharma, A., Mohanta, S.S., Brockmann, A., Dhandapany, P.S., Olsson, S.B., 2020. A field-based quantitative analysis of sublethal effects of air pollution on pollinators. Proc Natl Acad Sci U S A 117 (34), 20653–20661.

Trede, N. S., Langenau, D. M., Traver, D., Look, A. T., & Zon, L. I. (2004). The use of zebrafish to understand immunity. *Immunity, 20*(4), 367-379. 10.1016/S1074-7613(04)00084-6.

Villeneuve, D.L., Crump, D., Garcia-Reyero, N., Hecker, M., Hutchinson, T.H., LaLone, C. A., Landesmann, B., Lettieri, T., Munn, S., Nepelska, M., Ottinger, M.A., Vergauwen, L., Whelan, M., 2014. Adverse outcome pathway (AOP) development I: strategies and principles. Toxicol. Sci. 142 (2), 312–320. https://doi.org/10.1093/toxsci/kfu199.

Volta, A., Sforzini, S., Camurati, C., Teoldi, F., Maiorana, S., Croce, A., et al. (2020). Ecotoxicological effects of atmospheric particulate produced by braking systems on aquatic and edaphic organisms. *Environ. Int., 137*, 105564. doi:10.1016/j.envint.2020.105564.

Wang, J.S., Tseng, C.Y., Chao, M.W., 2017a. Diesel exhaust particles contribute to endothelia apoptosis via autophagy pathway. Toxicol. Sci. 156 (1), 72–83. https://doi.org/10.1093/toxsci/kfw237.

Wang, M., Nie, Y., Liu, Y., Dai, H., Wang, J., Si, B., Yang, Z., Cheng, L., Liu, Y., Chen, S., Xu, A., 2019. Transgenerational effects of diesel particulate matter on Caenorhabditis elegans through maternal and multigenerational exposure. Ecotoxicol. Environ. Saf. 170, 635–643. https://doi.org/10.1016/j.ecoenv.2018.12.027.

Wang, X., Chen, M., Zhong, M., Hu, Z., Qiu, L., Rajagopalan, S., Ying, Z., 2017b. Exposure to concentrated ambient PM2.5 shortens lifespan and induces inflammation-associated signaling and oxidative stress in Drosophila. Toxicol. Sci. 156 (1), 199–207. https://doi.org/10.1093/toxsci/kfw240.

Wu, Q., Han, X., Wang, D., Zhao, F., Wang, D., 2017. Coal combustion related fine particulate matter (PM2.5) induces toxicity in Caenorhabditis elegans by dysregulating microRNA expression. Toxicol. Res. (Camb) 6 (4), 432–441. https://doi.org/10.1039/c7tx00107j.

Yan, C., Wu, X., Cao, X., Li, M., Zhou, L., Xiu, G., Zeng, J., 2021. In vitro and in vitro toxicity study of diesel exhaust particles using BEAS-2B cell line and the nematode Caenorhabditis elegans as biological models. Environ. Sci. Pollut. Res. Int. 28 (43), 60704–60716. https://doi.org/10.1007/s11356-021-14908-0.

Yang, R., Rui, Q., Kong, L., Zhang, N., Li, Y.u., Wang, X., Tao, J., Tian, P., Ma, Y., Wei, J., Li, G., Wang, D., 2016. Metallothioneins act downstream of insulin signaling to regulate toxicity of outdoor fine particulate matter (PM2.5) during Spring Festival in Beijing in nematode Caenorhabditis elegans. Toxicol. Res. (Camb) 5 (4), 1097–1105. https://doi.org/10.1039/c6tx00022c.

Yuan, Q., Chen, Y., Li, X., Zhang, Z., Chu, H., 2019. Ambient fine particulate matter (PM2.5) induces oxidative stress and pro-inflammatory response via up-regulating the expression of CYP1A1/1B1 in human bronchial epithelial cells in vitro. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 839, 40–48. https://doi.org/10.1016/j.mrgentox.2018.12.005.

Zhang, H., Yao, Y., Chen, Y., Yue, C., Chen, J., Tong, J., Chen, T., 2016. Crosstalk between AhR and wnt/beta-catenin signal pathways in the cardiac developmental toxicity of PM2.5 in zebrafish embryos. Toxicology 355–356, 31–38. https://doi.org/10.1016/j.tox.2016.05.014.

Zhang, Y., Jia, Z., Rajendran, R.S., Zhu, C., Wang, X., Liu, K., Cen, J., 2021. Exposure of particulate matter (PM10) induces neurodevelopmental toxicity in zebrafish embryos. Neurotoxicology 87, 208–218. https://doi.org/10.1016/j.neuro.2021.10.004.

Zhang, Y., Li, S., Li, J., Han, L., He, Q., Wang, R., Wang, X., Liu, K., 2018. Developmental toxicity induced by PM2.5 through endoplasmic reticulum stress and autophagy pathway in zebrafish embryos. Chemosphere 197, 611–621.

Zhao, Y., Jin, L., Chi, Y., Yang, J., Zhen, Q., Wu, H., 2019. Fine Particulate matter leads to unfolded protein response and shortened lifespan by inducing oxidative stress in C. elegans. Oxid. Med. Cell. Longevity 2019, 1–10.