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Wing morphological changes in *Drosophila melanogaster* exposed to Bisphenol-A and Acrylamide

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Abstract. *Zubaidah S, Fauzi A, Setiawan D, Mulyati Y, Choirunisa' N, Zahrah NA. 2025. Wing morphological changes in* Drosophila melanogaster *exposed to Bisphenol-A and Acrylamide. Biodiversitas 26: 4567-4576.* Bisphenol-A (BPA) and acrylamide are two contaminants commonly found in processed food and beverage products. This study aimed to evaluate the impact of acute exposure to BPA and acrylamide contaminants on changes in *Drosophila melanogaster* wing morphology. The use of wings is based on their sensitivity to environmental changes and is one of the important organs for locomotion and mating in flies. In this study, the wild-type, black, and white strains of *D. melanogaster* were cultured for two generations to observe the acute exposure to these two contaminants. The statistical analysis showed that acute exposure to BPA and acrylamide for two generations did not correlate strongly with wing length and width changes in the population of the three *D. melanogaster* strains ($p<0.01$). We suspect that the lack of correlation may be influenced by the non-monotonic nature of BPA, the detoxification pathway of acrylamide, and exposure studies that are still limited to acute exposure. Of all flies observed, no wing morphological changes were found in the control group. However, <15% of the group exposed to BPA and acrylamide showed wing deformities (e.g., curled, wrinkled, and notched, broken wing veins, detachment, failure in growth, and imperfections in the formation of wing edges or wing cells). The results of this study require further studies, especially on other aspects of the study and exposure over several generations, long enough to see the impact of chronic exposure to BPA and acrylamide over the next few generations.

Keywords: Environmental contaminant, food contaminant, fruit flies, wing morphology

INTRODUCTION

Bisphenol-A (2, 2-bis (4-hydroxyphenyl) propane, BPA) is widely used in the manufacture of polycarbonate plastics and epoxy resins for food and beverage packaging. Demand for BPA in 2022 is expected to reach around 34 million tons, representing a 455-fold increase over the past 30 years (Wang et al. 2025) and also expected to increase by about 4% until 2030 (Tsai 2023). In addition to BPA, acrylamide is another chemical contaminant frequently found in heat-processed food, such as those that are fried, baked, or grilled (Adimas et al. 2024). This contaminant has been found in excess in biscuits, processed chicken, and children's snacks (Bonucci et al. 2024), high-temperature cooked vegetables, and processed potato chips (Yu et al. 2023). In Indonesia, about 17.4% of the population has ingested acrylamide above the safe weekly intake limit of 18.2 µg/kg-bw/week (Pratama and Jacxsens 2019).

Food sources are not the only source of BPA and acrylamide. Indoor dust, surface water, sediments, and sewage sludge contain BPA, contaminating the environment (Molina et al. 2021; Sharma et al. 2021). Acrylamide's high solubility and mobility in water can contaminate surface and groundwater supplies (Tepe and Çebi 2019; Naiel et al. 2023). Thus, BPA and acrylamide exposure are also environmental. BPA and acrylamide health risks are well known. BPA is linked to polycystic ovarian syndrome (PCOS), decreased sperm count and quality (Matuszczak et al. 2019; Praveen et al. 2020), and cardiovascular diseases like hypertension and vascular congestion (Nomiri et al. 2019). In addition, acrylamide metabolites like glycidamide are linked to cardiovascular risk factors like high glucose and lipid levels, blood pressure, and obesity (Mérida et al. 2024).

Both compounds are toxic to animals in lab studies. BPA causes DNA damage in *Xenopus laevis* (Ge et al. 2021), retinal layer changes in *Danio rerio* Westaquarium strain larvae (Volz et al. 2024), outer bone structure changes in Wistar rats (Prasse et al. 2022), and wing deformities in *Drosophila melanogaster* (Zahrah et al. 2025). Acrylamide caused zebrafish morphological malformations (Park et al. 2021) and D. melanogaster larval distance (Kharomah et al. 2025b). Natural organisms are constantly exposed to contaminants through water or living environments. Exposure over generations can have cumulative biological effects (DeCourten et al. 2020).

Individual responses vary depending on contaminants (Smorodinskaya et al. 2023), sex (Begum et al. 2021), and strain (Ramadhan et al. 2025). To assess biological risks from BPA and acrylamide acute exposure, generation, concentration, sex, and strain must be considered.

Drosophila melanogaster is a widely used model organism in toxicology due to its short life cycle, low maintenance costs, and well-mapped genome (Rovik et al. 2022; Rand et al. 2023; Kusmintarsih et al. 2025). *Drosophila* is commonly used as a model organism to study human diseases because about 60-70% of human disease-causing genes have homologs in *Drosophila* (Koehler and Huber 2023). In addition, many key signaling pathways involved in development and metabolism are conserved between *Drosophila* and humans (Megaly et al. 2024).

Wings are one of the sensitive organs that can react to different environmental changes (Szabla et al. 2024). Because of this sensitivity, the wing can be used as an indicator of environmental changes in Drosophila. Morphological changes in the wings can reflect developmental instability caused by environmental stress, including exposure to contaminants (Fatmawati et al. 2023; Lewandowska-Wosik and Chudzińska 2024). Wings are essential for flight and overall fitness (Dong et al. 2020). In males, they also produce courtship songs critical for mating (Houot et al. 2017). Thus, morphological changes in wings can impair mating success and lead to population decline.

Previous research found that BPA alters Drosophila wings (Zahrah et al. 2025). However, the study only covered one generation. Additionally, acrylamide-induced wing morphological changes have not been studied. Thus, this study examined how acute BPA and acrylamide exposure affected *D. melanogaster* wing shape. This study should help the health sector by revealing the effects of acute BPA and acrylamide exposure disorders, developing related therapies, and regulating safe consumption. Ecological toxicology also benefits from understanding how these contaminants affect Drosophila wing morphology. This study hypothesizes that exposure to BPA and acrylamide correlates with morphological changes in fly wings.

MATERIALS AND METHODS

Drosophila melanogaster preparation

This study used three strains of *D. melanogaster,* wild-type (N), black (b), and white (w). *Drosophila melanogaster* samples were maintained under optimal conditions, 24°C temperature, 60% humidity, and a 12:12 h light-dark cycle. Flies were cultured in standard media consisting of rajamala banana (*Musa ×paradisiaca* cv. Rajamala), fermented cassava (Manihot esculenta), and brown sugar in a ratio of 7:2:1 grams. All ingredients are blended together and then cooked for 45 minutes, based on previous research (Fauzi et al. 2020).

Research design

This study comprised two experiments in which BPA and acrylamide were mixed into the *D. melanogaster* culture medium for oral exposure. A true experimental pretest-posttest control group design was used (Figure 1), with control (untreated) and experimental groups. BPA was tested at 0.25 and 0.50 mg/mL, while acrylamide was given at 24 and 48 mg/kg bw/day, following previous studies (Kharomah et al. 2025a; Ramadhan et al. 2025). Each treatment was repeated four times, referring to the calculation formula of group randomized design, with five male-female pairs per replicate. At each concentration, 360 flies were observed, totaling around 1,440 flies across all treatments. The experiment was carried out for two generations to assess the acute effects of BPA and acrylamide.

Analysis of changes in wing morphology

Flies were anaesthetized using chloroform, then a pair of wings was removed with a needle or tweezers. Wing abnormalities were examined with an Olympus SZ61 trinocular stereo microscope, and then the analysis results were captured with an Olympus EP50 camera. After that, the length and width of the right and left wings were measured with Epview software, which had been calibrated beforehand with a microscope. The reference for measuring wing length and width was adapted from previous research (Lack et al. 2016) (Figure 2). For wing length measurement, a straight line was drawn from the anterior cross vein across the medial vein. Meanwhile, the width measurement was measured by drawing b line from the distal length of the vein across the posterior cross vein.

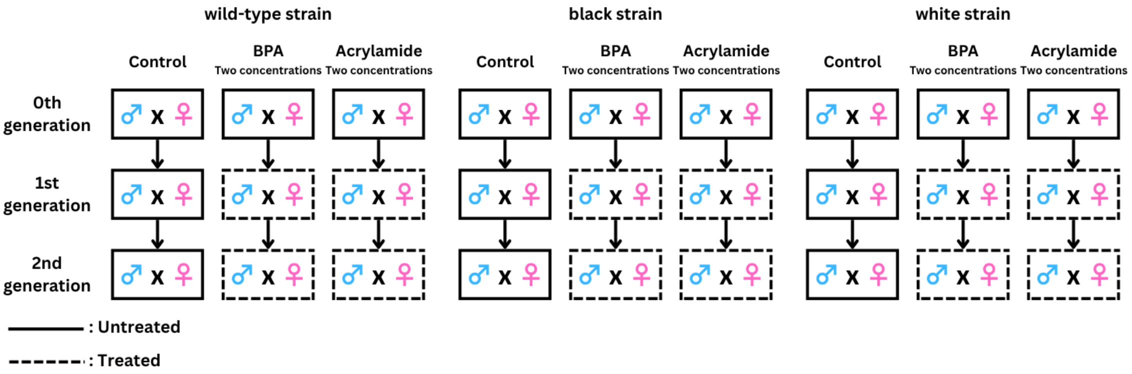

Figure 1. Breeding scheme of *Drosophila melanogaster*

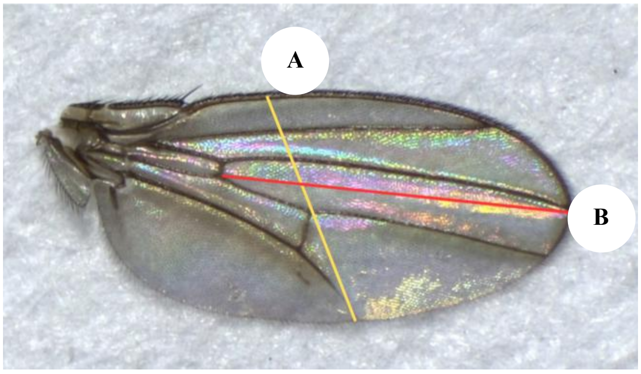

Figure 2. Measurement of *Drosophila melanogaster* wings. A. Width, B. Length

Data analysis

The experimental data were analyzed using Statistical Package for the Social Sciences (SPSS) 26 and Kendall's Tau correlation test. This test does not assume linearity or normality, making it appropriate for analyzing rank-based morphological changes and their relationship with exposure variables such as concentration, sex, strain, and generation. Then, the correlation results were interpreted based on the correlation coefficient, according to Schober et al. (2018). Meanwhile, changes in wing morphology were analyzed descriptively and quantitatively, which was supported by several previous reference sources (Lack et al. 2016; Fatmawati et al. 2023; Sun et al. 2023; Sorensen et al. 2024). Significance in this study refers to the results of Kendall's Tau correlation analysis.

RESULTS AND DISCUSSION

Interpretation of the correlation between generation and sex

Correlation between generation and sex of BPA acute exposure

Table 1 shows a significant correlation (τ: 0.794; p<0.01) between *D. melanogaster* wing length and width, confirming that acute BPA exposure impacts both dimensions. Although significant, BPA exposure over two generations does not significantly correlate with wing length (τ: 0.113; p<0.01) or width (τ: 0.057; p<0.01), suggesting a low contribution to variability. Sex was moderately correlated with wing length (τ: 0.464; p<0.01) and width (τ: 0.357; p<0.01), confirming gender differences in size. No relationship was found between generation and sex (τ<0.001; p>0.999).

Correlation between generation and sex of acrylamide acute exposure

Table 2 shows a strong relationship between wing width and length in *D. melanogaster* exposed to acrylamide (τ: 0.713; p<0.01). Intergenerational acrylamide exposure has a weak negative relationship with wing length (τ: -0.146; p<0.01) and width (τ: -0.236; p<0.01), suggesting that increased exposure may reduce the size. Despite their moderate strength, these statistically significant relationships show acrylamide may accumulate and impact phenotypic traits. The study found a moderate negative connection between sex and wing length (τ: -0.402; p<0.01) and a weakened relationship with wing width (τ: -0.247; p<0.01). Males have smaller wings than females. No connection was found between generation and sex (τ: 0.001; p>0.999).

Interpretation of the correlation between concentration and strain

Correlation between concentration and strain of BPA acute exposure

Table 3 reveals that BPA levels and strain variation (wild-type, black, white) did not affect wing morphometric size sufficiently. Strain showed minimal correlation on wing length (τ: 0.078; p<0.01) and width (τ: 0.138; p<0.01), showing minimal genetic influence on size variation. High BPA concentrations had little impact on wing shape, with only small associations with wing length (τ: 0.072; p<0.01) and width (τ: 0.044; p<0.01). Despite significant (p<0.01), modest coefficients suggest minimal correlation between genetic and environmental factors and short-term exposure. All groups showed a strong connection between wing length and width (τ: 0.794; p<0.01).

Table 1. Correlation between generation and sex of BPA acute exposure

		Length	Width	Generation	Sex
Length	Correlation coefficient	1	0.794**	0.113**	0.464**
	Sig. (2-tailed)		0.000	0.000	0.000
	N	2160	2160	2160	2160
Width	Correlation coefficient	0.794**	1	0.057**	0.357**
	Sig. (2-tailed)	0.000		0.008	0.000
	N	2160	2160	2160	2160
Generation	Correlation coefficient	0.113**	0.057**	1	0.000
	Sig. (2-tailed)	0.000	0.008		1.000
	N	2160	2160	2160	2160
Sex	Correlation coefficient	0.464**	0.357**	000	1
	Sig. (2-tailed)	0.000	0.000	1.000	
	N	2160	2160	2160	2160

Note: **: Correlation is significant at the 0.01 level (2-tailed)

Table 2. Correlation between generation and sex of acrylamide acute exposure

		Length	Width	Generation	Sex
Length	Correlation coefficient	1	0.713**	-0.146**	-0.402**
	Sig. (2-tailed)		0.000	0.000	0.000
	N	2160	2160	2160	2160
Width	Correlation coefficient	0.713**	1	-0.236**	-0.247**
	Sig. (2-tailed)	0.000		0.000	0.000
	N	2160	2160	2160	2160
Generation	Correlation coefficient	-0.416**	-0.236**	1	0.000
	Sig. (2-tailed)	0.000	0.000		1.000
	N	2160	2160	2160	2160
Sex	Correlation coefficient	-0.402**	-.0.247**	000	1
	Sig. (2-tailed)	0.000	0.000	1.000	
	N	2160	2160	2160	2160

Note: **: Correlation is significant at the 0.01 level (2-tailed)

Table 3. Correlation between concentration and strain of BPA acute exposure

		Strain	Concentration	Length	Width
Strain	Correlation coefficient	1	0.000	0.078**	0.138**
	Sig. (2-tailed)		1.000	0.000	0.000
	N	2160	2160	2160	2160
Concentration	Correlation coefficient	0.000	1	0.072**	0.044*
	Sig. (2-tailed)	1.000		0.001	0.039
	N	2160	2160	2160	2160
Length	Correlation coefficient	0.078**	0.072**	1	0.794**
	Sig. (2-tailed)	0.000	0.001		0.000
	N	2160	2160	2160	2160
Width	Correlation coefficient	0.138**	0.044*	0.794**	1
	Sig. (2-tailed)	0.000	0.039	0.000	
	N	2160	2160	2160	2160

Note: *: Correlation is significant at the 0.05 level (2-tailed), **: Correlation is significant at the 0.01 level (2-tailed)

Figure 3 shows that the control and BPA experimental groups have similar wing lengths and widths. In addition, in line with the statistical test results in Table 3, wild-type, black, and white strains strongly correlate wing length and width (τ: 0.794; p<0.01), meaning that the longer the fly's wing size, the greater its width.

Correlation between concentration and strain of acrylamide acute exposure

Table 4 indicates no significant correlation was found between fly strains and wing length (τ: 0.031; p>0.01) or breadth (τ: 0.034; p>0.01) following acrylamide exposure. The study found no significant link between acrylamide concentration and wing length (τ: 0.016; p>0.01), although a weak negative correlation (τ: -0.084; p<0.01) indicated a slight decrease in wing width with higher concentrations. The effect is small, but it suggests marginal morphometric changes, particularly in wing breadth. A consistently substantial connection (τ: 0.713; p<0.01) exists between wing length and width.

Based on statistical tests, it should be emphasized that the correlation values contained therein do not have significant ecological meaning because the correlation level is very weak.

Figure 4 shows that the control and acrylamide experimental groups have similar wing lengths and widths. In addition, in line with the statistical test results in Table 4, wild-type, black, and white strains strongly correlate wing length and width (τ: 0.713; p<0.01), meaning that the longer the fly's wing size, the greater its width.

Correlation between the effects of acute exposure to BPA on wing length and width

BPA exposure caused wing morphological changes in the three *D. melanogaster* strains; however, statistical analysis showed no strong connection between BPA and wing length or width (Table 3). This implies that 0.25 and 0.50 mg/mL concentrations do not affect the population. BPA's non-monotonic dose-response behavior, often forming a “U”-shaped curve (Kim et al. 2019; Peters et al. 2024), may explain this result, as the tested doses may be in the middle of the “U” curve or even at the maximum point where there is no significant effect.

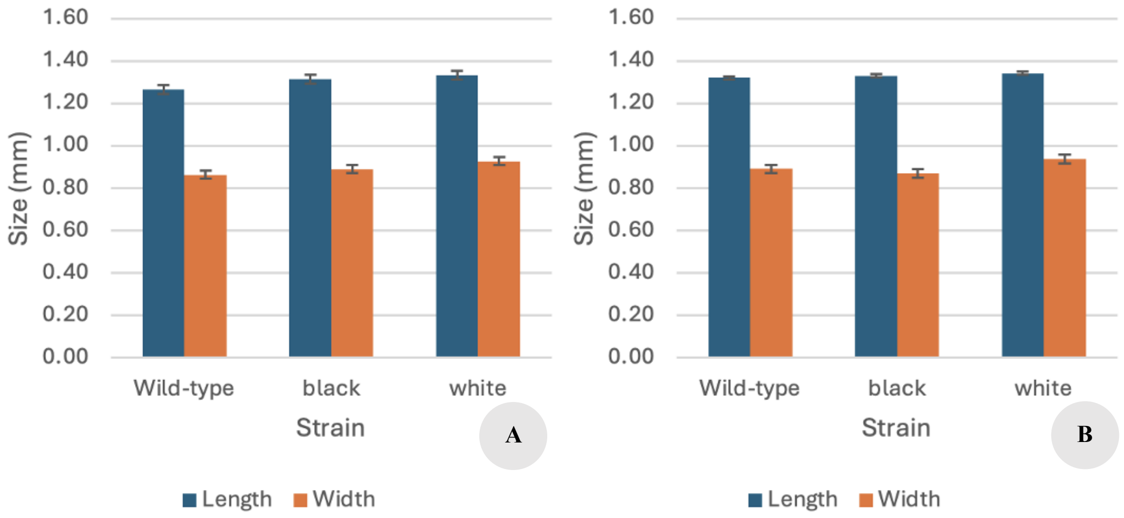

Figure 3. Comparison of wing length and width of the three *Drosophila melanogaster* strains exposed to BPA. A. Control group, B. Treatment group

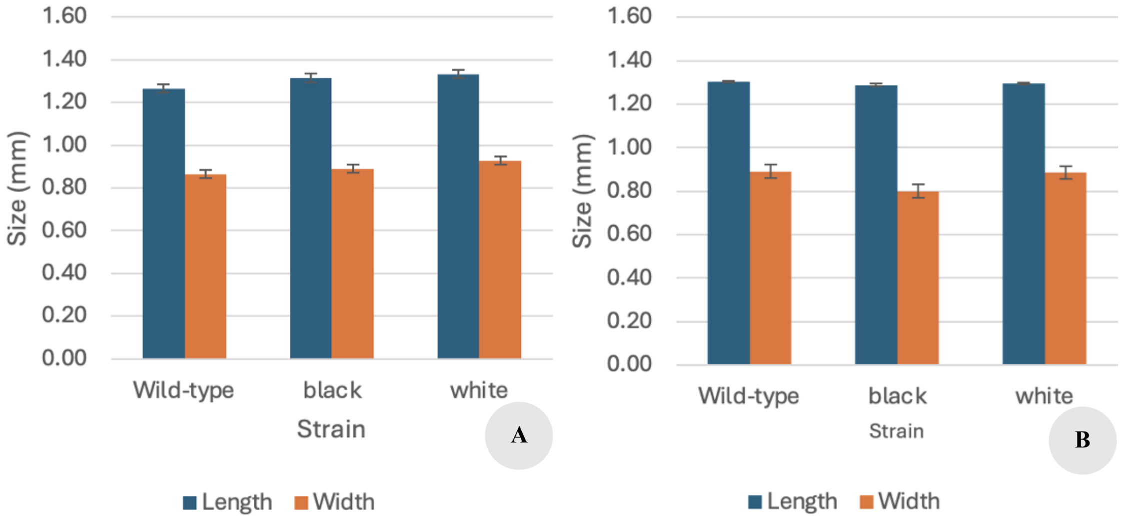

Figure 4. Comparison of wing length and width of the three *Drosophila melanogaster* strains exposed to acrylamide. A. Control group, B. Treatment group

Table 4. Correlation between concentration and strain of acrylamide acute exposure

		Strain	Concentration	Length	Width
Strain	Correlation coefficient	1	0.000	0.031	0.034
	Sig. (2-tailed)		1.000	0.154	0.113
	N	2160	2160	2160	2160
Concentration	Correlation coefficient	0.000	1	0.016	-0.084**
	Sig. (2-tailed)	1.000		0.470	0.000
	N	2160	2160	2160	2160
Length	Correlation coefficient	0.031	0.016	1	0.713**
	Sig. (2-tailed)	0.154	0.47		0.000
	N	2160	2160	2160	2160
Width	Correlation coefficient	0.034	-0.084**	0.713	1
	Sig. (2-tailed)	0.113	0.000	0.000	
	N	2160	2160	2160	2160

Note: **: Correlation is significant at the 0.01 level (2-tailed)

In addition to concentration-related effects, several studies have shown that BPA may not significantly impact certain biological parameters when exposure duration is short. For example, short-term BPA exposure did not impair LH/hCG-induced steroidogenic potential in human granulosa or rat Leydig cells (Roy et al. 2023). Another study found that short-term BPA exposure did not influence nursing rabbits' body weight, feed intake, or milk production (Hao et al. 2024). BPA exposure for two months did not significantly impact sheep femur metabolism, macro- and microstructure, or biomechanical behavior (Brankovič et al. 2022). However, more research is needed to determine how concentration and exposure period affect the test organism.

Correlation between the effects of acute exposure to acrylamide on wing length and width

In this study, acrylamide concentrations (24 and 48 mg/kg bw/day) exceeded the oral exposure limit (<0.5 μg/L or $<1.43\times10^{-5}$ mg/kg bw/day), but showed little association with wing length or width among *D. melanogaster* strains (Table 4). Acrylamide may activate detoxification mechanisms at high doses. Wu et al. (2023), found that acrylamide increases GSTP1, GSH, and CYP2E1 activity, helping the body eliminate toxins. Acrylamide or its metabolites binding to GSTP1 and CYP2E1 may balance toxicity and detoxification, limiting morphological alterations. These mechanisms may explain why wing morphometrics did not change despite large doses in the study. In addition, short-term acrylamide exposure does not boost ROS generation (Johansson et al. 2024). Meanwhile, *D. melanogaster* wing disc ROS produces severe mitochondrial abnormalities (Mumbauer et al. 2019). In this case, it is suspected that acute acrylamide exposure may not have caused ROS accumulation; therefore, the cell division cycle and fly wing growth continued properly.

Diversity of wing morphology changes of 0th to 2nd generation *Drosophila melanogaster* strains

Despite no statistical relationships, under fifteen percent of the treatment population had wing morphological changes. The control groups showed no variation (Figure 5). The treatment groups had three main damage categories. First, curved, wrinkled, notched wings. Damage can be small or serious. A single area of minor damage curls or wrinkles the wingtip or edges without affecting the structure (Figure 6.F). Major damage causes widespread deformation in both wings, affecting wing shape compared to controls (Figure 7.A). Second, vein rupture, separation, or failure at growth. These are small or major based on severity and the region affected. Minor injury affects vein growth in one wing or a small vein portion with limited distribution (Figure 7.E). Both wings have severe damage, indicating structural vein disruption (Figure 7.D). Third, imperfect wing edges. This damage is minor or major. Major damage comprises vein fractures that change wing shape (Figure 6.C), while minor damage involves deformed wing edges without interior vein damage (Figure 8.E).

Wing alterations differ greatly across individuals who are exposed. Many wings showed vein fractures, indentations, and uneven edges. Vigneron et al. (2019) suggest that genetic and phenotypic adaptation increase pollution tolerance and explain variability. These effects may be temporary and population-specific, making their relevance unclear in our investigation.

Figure 5. Wing morphology in the control group of female wild-type *Drosophila melanogaster* strain

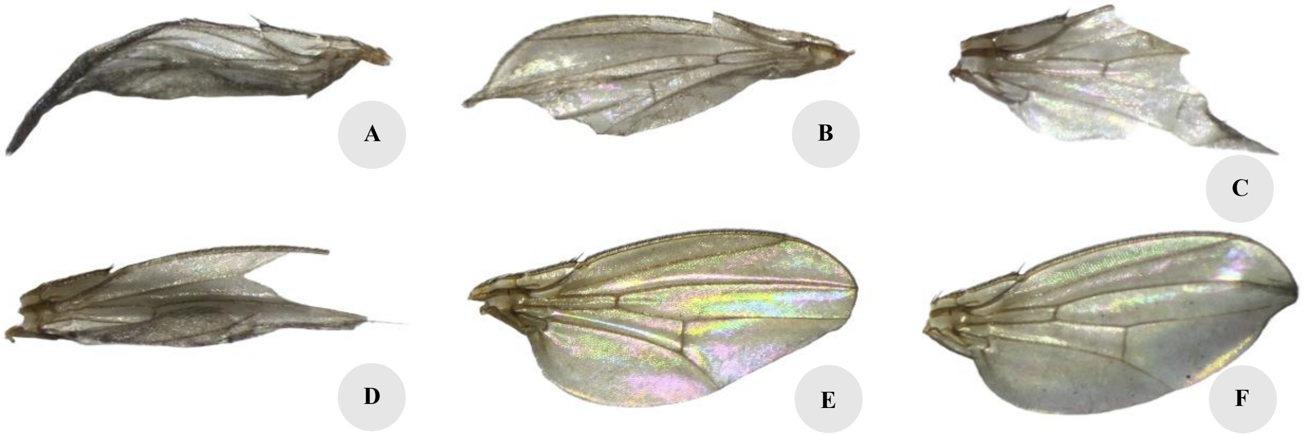

Figure 6. Diversity changes in wing morphology of *Drosophila melanogaster* wild-type strain. A-D: Wings in the BPA exposure group. E-F: Wings in the acrylamide exposure group. A, B, F: Damaged wings in curled, wrinkled, and notched conditions. C-D: Damage to the wing veins, such as fracture, detachment, or failure of vein growth. C, E: Defective wing in terms of incomplete wing margin formation or wing cells

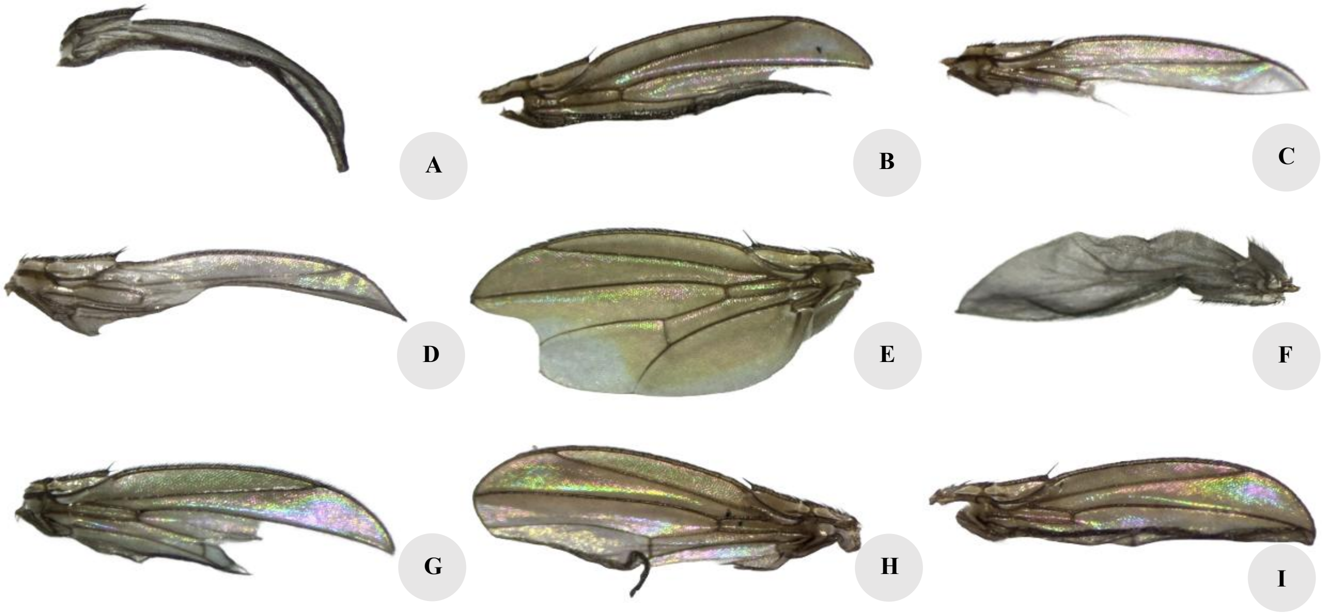

Figure 7. Diversity changes in wing morphology of *Drosophila melanogaster* black strain. A-E: Wings in the BPA exposure group. F-I: Wings in the acrylamide exposure group. A, B, F, H, I: Damaged wings in curled, wrinkled, and notched conditions. C-E, G: Damage to the wing veins, such as fracture, detachment, or failure of vein growth. E, G: Defective wing in terms of incomplete wing margin formation or wing cells

Figure 8. Diversity changes in wing morphology of *Drosophila melanogaster* white strain. No obvious wing damage was found in the BPA treatment group. A-F: Wings in the acrylamide exposure group. A-D, F: Damaged wings in curled, wrinkled, and notched conditions. A-B: Damage to the wing veins, such as fracture, detachment, or failure of vein growth. E: Defective wing in terms of incomplete wing margin formation or wing cells

BPA and acrylamide exposure in *D. melanogaster* varies by strain and generation. Descriptively, the second-generation responses varied considerably. Wrinkles and faulty margins are observed in wild-type strains exposed to BPA at 0.25 mg/mL. Whereas the first generation showed only defects. Second-generation wild-type strains given 48 mg/kg BW/day acrylamide displayed broken veins, notched wings, and imperfect wing edges. Major wing alterations did not affect the first generation. The damage to the first and second generations is quantitatively less than 15%.

Wild-type strains have more wing structure variation than white strains. Acrylamide exposure in white strains causes more morphological changes than BPA. The white and wild-type strains had fewer wing morphological changes and fewer damage types than the black strain. Black strains may be more sensitive to pollutants than wild-type and white strains. Miyagi et al. (2015) determined that complicated quantitative loci continue to change *D. melanogaster*'s ecological features, including wing shape. Unknown processes sustain population variation. Defective *Drosophila* strains can harm and control surrounding genes pleiotropically, like the apterous gene, which influences wing development. A mutation in the apterous gene near the black strain's mutation locus may explain white and black strain responses.

BPA causes wing distortion in wild-type *D. melanogaster* (Begum et al. 2021) and damage or size reduction in other strains (Zahrah et al. 2025). BPA downregulates wingless (Wg), decapentaplegic (Dpp), and vestigial (Vg) genes (Begum et al. 2021). Wg depends on wing specification and boundary development (Gracia-Latorre et al. 2022; Rosales-Vega et al. 2023). Dpp might not directly affect late-stage wing disc formation (Akiyama and Gibson 2015), but it regulates cell division and tissue size to promote expansion (Barrio and Milán 2017). Dpp, Wg, and Vg signals maintain wing structure (Fan et al. 2021).

As with BPA, acrylamide is a fruit fly wing disc cell mutagen and recombinogen (Tripathy et al. 1991). Acrylamide causes mitochondrial malfunction and cell death by disrupting bioenergetic and respiratory performance (Farodoye et al. 2024). Energy for growth and development can be reduced by mitochondrial malfunction (Madan et al. 2022). Mitochondrial diseases can impede *D. melanogaster* wing growth, leading in broken veins, uneven wing margins, and wrinkled or notched wings. Broken veins, irregular wing margins, and wrinkled or notched wings can result from mitochondrial disorders in *D. melanogaster*. This chemical attaches to DNA via acrylamide's α,β-unsaturated carbonyl group, causing oxidative stress and morphological changes (Prasad and Muralidhara 2012; Ou et al. 2020).

Wing defects could impede Drosophila ecological adaptation. They can impede flight and mating (Davis et al. 2018), making it harder for predator avoidance, food acquisition, and habitat change. Males with damaged wings are less likely to mate, decreasing population growth. Inability to fly (Rajabi et al. 2020) and move around, as well as escape from unfavorable conditions (Halder et al. 2025), can reduce lifespan. These influences may affect population dynamics.

In conclusion, the statistical analysis showed no effect of two generations of contaminant exposure on wing length or width. However, several *D. melanogaster* wild-type, black, and white strains showed bent, wrinkled, notched wings, broken veins, and imperfect wing edges. Early development gene disruption or DNA damage in imaginal disc cells may cause wing abnormalities after BPA and acrylamide exposure, showing environmental mutagenesis effects. This result suggests that BPA's non-monotonic response, acrylamide detoxification, or limited exposure period may induce non-linear effects. This study has several limitations. First, the Alula wing cell may be separated during wing removal. Therefore, measurements are only based on predefined length and width. Second, only two concentrations of each contaminant are used. Third, cross-generation analysis only uses two generations and does not examine contaminants on future generations. Future studies should examine multi-generational effects, gene expression patterns, analysis using geometric morphometrics, and ecological consequences to understand how environmental toxins affect insect development, reproduction, and population stability.

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ETHICAL APPROVAL

This study was conducted after obtaining approval from the ethics committee of the Institute of Biosciences, Universitas Brawijaya, Malang, Indonesia, No. 136-KEP-UB-2024.

REFERENCES

Adimas MA, Abera BD, Adimas ZT, Woldemariam HW, Delele MA. 2024. Traditional food processing and Acrylamide formation: A review. Heliyon 10 (9): e30258. DOI: 10.1016/j.heliyon.2024.e30258.

Akiyama T, Gibson MC. 2015. Decapentaplegic and growth control in the developing *Drosophila* wing. Nature 527 (7578): 375-378. DOI: 10.1038/nature15730.

Barrio L, Milán M. 2017. Boundary Dpp promotes growth of medial and lateral regions of the *Drosophila* wing. Elife 6: 1-20. DOI: 10.7554/eLife.22013.

Begum M, Paul P, Das D, Chakraborty K, Bhattacharjee A, Ghosh S. 2021. Genes regulating development and behavior exhibited altered expression in *Drosophila melanogaster* exposed to bisphenol A: Use of real-time quantitative PCR (qRT-PCR) and droplet digital PCR (ddPCR) in genotoxicity study. Environ Sci Pollut Res 28 (6): 7090-7104. DOI: 10.1007/s11356-020-10805-0.

Bonucci A, Urbani S, Servili M, Selvaggini R, Daidone L, Dottori I, Sordini B, Veneziani G, Taticchi A, Esposto S. 2024. Baby Foods: 9 out of 62 exceed the reference limits for Acrylamide. Foods 13 (17): 2690. DOI: 10.3390/foods13172690.

Brankovič J, Leskovec J, Šturm S, Cerkvenik-Flajs V, Šterpin S, Osredkar J, Pogorevc E, Antolinc D, Vrecl M. 2022. Experimental exposure to bisphenol a has minimal effects on bone tissue in growing rams: A Preliminary Study. Animals 12 (17): 2179. DOI: 10.3390/ani12172179.

Davis SM, Thomas AL, Liu L, Campbell IM, Dierick HA. 2018. Isolation of aggressive behavior mutants in drosophila using a screen for wing damage. Genetics 208 (1): 273-282. DOI: 10.1534/genetics.117.300292.

DeCourten BM, Forbes JP, Roark HK, Burns NP, Major KM, White JW, Li J, Mehinto AC, Connon RE, Brander SM. 2020. Multigenerational and transgenerational effects of environmentally relevant concentrations of endocrine disruptors in an estuarine fish model. Environ Sci Technol 54 (21): 13849-13860. DOI: 10.1021/acs.est.0c02892.

Dong W, Gao Y, Zhang X, Moussian B, Zhang J. 2020. Chitinase 10 controls chitin amounts and organization in the wing cuticle of Drosophila. Insect Sci 27 (6): 1198-1207. DOI: 10.1111/1744-7917.12774.

Fan W, Luo D, Zhang J, Wang D, Shen J. 2021. Vestigial suppresses apoptosis and cell migration in a manner dependent on the level of JNK-Caspase signaling in the *Drosophila* wing disc. Insect Sci 28 (1): 63-76. DOI: 10.1111/1744-7917.12762.

Farodoye OM, Otenaike TA, Loreto JS, Adedara AO, Silva MM, Barbosa NV, Rocha JBT da, Abolaji AO, Loreto ELS. 2024. Evidence of acrylamide-induced behavioral deficit, mitochondrial dysfunction and cell death in *Drosophila melanogaster.* Comp Biochem Physiol Part C Toxicol Pharmacol 284: 109971. DOI: 10.1016/j.cbpc.2024.109971.

Fatmawati D, Khoiroh D, Zubaidah S, Susanto H, Agustin M, Fauzi A. 2023. Wing morphological changes of *Drosophila melanogaster* exposed with lead in nine generations. AIP Conf Proc 2634: 020041. DOI: 10.1063/5.0111882

Fauzi A, Zubaidah S, Susanto H. 2020. The study of larva and adult behavior of *Drosophila melanogaster*: Do strains affect behavior? AIP Conf Proc 2231: 040014. DOI: 10.1063/5.0002429.

Ge Y, Ren F, Chen L, Hu D, Wang X, Cui Y, Suo Y, Zhang H, He J, Yin Z, et al. 2021. Bisphenol A exposure induces apoptosis and impairs early embryonic development in *Xenopus laevis*. Environ Pollut 280: 116901. DOI: 10.1016/j.envpol.2021.116901.

Gracia-Latorre E, Pérez L, Muzzopappa M, Milán M. 2022. A single WNT enhancer drives specification and regeneration of the *Drosophila* wing. Nat Commun 13 (1): 4794. DOI: 10.1038/s41467-022-32400-2.

Halder S, Bhore U, Nandy B. 2025. Female-biased spontaneous dispersal in *Drosophila melanogaster* and sex-specific effect of nutrition and density therein. Oikos 2025 (6): 10920. DOI: 10.1111/oik.10920.

Hao J, Beng S, Ma Z, Xu H, Yang T, Yang Q, Wang Y, Zheng W, Ma Y, Zhang S. 2024. Short-term exposure of Bisphenol A deteriorates the quality of rabbit milk by impairing milk fat synthesis. Food Sci Nutr 12 (12): 10666-10679. DOI: 10.1002/fsn3.4561.

Houot B, Gigot V, Robichon A, Ferveur J-F. 2017. Free flight odor tracking in *Drosophila*: Effect of wing chemosensors, sex and pheromonal gene regulation. Sci Rep 7 (1): 40221. DOI: 10.1038/srep40221.

Johansson Y, Andreassen M, Hartsch M, Wagner S, Forsby A. 2024. Attenuated neuronal differentiation caused by acrylamide is not related to oxidative stress in differentiated human neuroblastoma SH-SY5Y cells. Food Chem Toxicol 187: 114623. DOI: 10.1016/j.fct.2024.114623.

Kharomah S, Ramadhan MJ, Zahrah NA, Kharomah S, Agustin M, Mirandah D, Fauzi A, Zubaidah S. 2025a. Impact of Acrylamide on crawling activity of three *Drosophila* Strains: Larval Speed and contraction number. IOP Conf Ser Earth Environ Sci 1439 (1): 012012. DOI: 10.1088/1755-1315/1439/1/012012.

Kharomah S, Zahrah NA, Ramadhan MJ, Kharomah S, Hayuana W, Fauzi A, Zubaidah S. 2025b. Adaptation of the exploratory larva observation method: Comparative strain analysis and Acrylamide Effect. BIO Web Conf 183: 01006. DOI: 10.1051/bioconf/202518301006.

Kim JK, Khan A, Cho S, Na J, Lee Y, Bang G, Yu W-J, Jeong J-S, Jee SH, Park YH. 2019. Effect of developmental exposure to bisphenol A on steroid hormone and vitamin D3 metabolism. Chemosphere 237: 124469. DOI: 10.1016/j.chemosphere.2019.124469.

Koehler S, Huber TB. 2023. Insights into human kidney function from the study of *Drosophila*. Pediatr Nephrol 38 (12): 3875-3887. DOI: 10.1007/s00467.

Kusmintarsih ES, Gemilang P, Nuryanto A, Mahmoud HHA, Ambarningrum TB. 2025. Phylogeny of Wolbachia in *Drosophila* mutant from Bandung, Indonesia. Biodiversitas 25 (12). 4894-4899. DOI: 10.13057/biodiv/d251226.

Lack JB, Yassin A, Sprengelmeyer QD, Johanning EJ, David JR, Pool JE. 2016. Life history evolution and cellular mechanisms associated with increased size in high-altitude *Drosophila*. Ecol Evol 6 (16): 5893-5906. DOI: 10.1002/ece3.2327.

Lewandowska-Wosik A, Chudzińska EM. 2024. Fluctuating asymmetry spotted wing *Drosophila* (Diptera: Drosophilidae) exposed to sublethal doses of acetamiprid and nicotine. Insects. 15 (9): 681. DOI: 10.3390/insects15090681.

Madan S, Uttekar B, Chowdhary S, Rikhy R. 2022. Mitochondria lead the way: Mitochondrial dynamics and function in cellular movements in development and disease. Front Cell Dev Biol 9: 781993. DOI: 10.3389/fcell.2021.781933.

Matuszczak E, Komarowska MD, Debek W, Hermanowicz A. 2019. The impact of Bisphenol A on fertility, reproductive system, and development: A review of the literature. Intl J Endocrinol 2019: 406871. DOI: 10.1155/2019/4068717.

Megaly Marvel, Turgambayeva A, Hallam RD, Foran G, Megaly Mark, Necakov A. 2024. Human diseases associated with notch signalling: Lessons from *Drosophila melanogaster.* Front Biosci 29 (6): 234. DOI: 10.31083/j.fbl2906234.

Mérida DM, Rey-García J, Moreno-Franco B, Guallar-Castillón P. 2024. Acrylamide exposure and cardiovascular risk: A systematic review. Nutrients 16 (24): 4279. DOI: 10.3390/nu16244279.

Miyagi R, Akiyama N, Osada N, Takahashi A. 2015. Complex patterns of cis-regulatory polymorphisms in ebony underlie standing pigmentation variation in *Drosophila melanogaster*. Mol Ecol 24 (23): 5829-5841. DOI: 10.1111/mec.13432.

Molina AM, Abril N, Lora AJ, Huertas-Abril PV, Ayala N, Blanco C, Moyano MR. 2021. Proteomic profile of the effects of low-dose bisphenol A on zebrafish ovaries. Food Chem Toxicol 156: 112435. 1-16. DOI: 10.1016/j.fct.2021.112435.

Mumbauer S, Pascual J, Kolotuev I, Hamaratoglu F. 2019. Ferritin heavy chain protects the developing wing from reactive oxygen species and ferroptosis. Plos Genet 15 (9): e1008396. DOI: 10.1371/journal.pgen.1008396.

Naiel MAE, Negm SS, Ghazanfar S, Farid A, Shukry M. 2023. Acrylamide toxicity in aquatic animals and its mitigation approaches: an updated overview. Environ Sci Pollut Res 30 (53): 113297-113312. DOI: 10.1007/s11356-023-30437-4.

Nomiri S, Hoshyar R, Ambrosino C, Tyler CR, Mansouri B. 2019. A mini review of bisphenol A (BPA) effects on cancer-related cellular signaling pathways. Environ Sci Pollut Res 26 (9): 8459-8467. DOI: 10.1007/s11356-019-04228-9.

Ou J, Zheng J, Huang J, Ho C-T, Ou S. 2020. Interaction of Acrylamide, Acrolein, and 5-Hydroxymethylfurfural with amino acids and DNA. J Agric Food Chem 68 (18): 5039-5048. DOI: 10.1021/acs.jafc.0c01345.

Park J-S, Samanta P, Lee S, Lee J, Cho J-W, Chun H-S, Yoon S, Kim W-K. 2021. Developmental and Neurotoxicity of Acrylamide to Zebrafish. Intl J Mol Sci 22 (7): 3518. DOI: 10.3390/ijms22073518.

Peters AE, Ford EA, Roman SD, Bromfield EG, Nixon B, Pringle KG, Sutherland JM. 2024. Impact of Bisphenol A and its alternatives on oocyte health: A scoping review. Hum Reprod Update 30 (6): 653-691. DOI: 10.1093/humupd/dmae025.

Prasad SN, Muralidhara. 2012. Evidence of acrylamide induced oxidative stress and neurotoxicity in *Drosophila melanogaster*-Its amelioration with spice active enrichment: Relevance to neuropathy. Neurotoxicology 33 (5): 1254-1264. DOI: 10.1016/j.neuro.2012.07.006.

Prasse T, Stratos I, Niehoff A, Christ H, Heck V, Meyer C, Mittlmeier T. 2022. Bisphenol A-related effects on bone morphology and biomechanical properties in an animal model. Toxics 10 (2): 86. DOI: 10.3390/toxics10020086.

Pratama Y, Jacxsens L. 2019. Quantitative risk assessment of acrylamide in Indonesian deep fried fritters as street food products. Curr Res Nutr Food Sci J 7 (3): 662-669. DOI: 10.12944/CRNFSJ.7.3.06.

Praveen S, Arjun AR, Prince SE. 2020. Toxic effects of bisphenol-A. Intl J Med Toxicol Leg Med 23 (3 and 4): 285-295. DOI: 10.5958/0974-4614.2020.00080.7.

Rajabi H, Dirks J-H, Gorb SN. 2020. Insect wing damage: Causes, consequences and compensatory mechanisms. J Exp Biol 223 (9): 1-8. DOI: 10.1242/jeb.215194.

Ramadhan MJ, Kharomah S, Kharomah S, Zahrah NA, Maghfiroh H, Fahmi MIN, Zubaidah S, Fauzi A. 2025. Negative geotaxis assay in three *Drosophila* strains consuming bisphenol A: Duration and number of climbing successes. IOP Conf Ser Earth Environ Sci 1439 (1): 012010. DOI: 10.1088/1755-1315/1439/1/012010.

Rand MD, Tennessen JM, Mackay TFC, Anholt RRH. 2023. Perspectives on the *Drosophila melanogaster* model for advances in toxicological science. Curr Protoc 3 (8): 1-39. DOI: 10.1002/cpz1.870.

Rosales-Vega M, Reséndez-Pérez D, Zurita M, Vázquez M. 2023. TnaA, a trithorax group protein, modulates wingless expression in different regions of the *Drosophila* wing imaginal disc. Sci Rep 13 (1): 15162. DOI: 10.1038/s41598-023-42169-z.

Rovik A, Daniwijaya EW, Supriyati E, Rahayu A, Kumalawati DA, Saraswati U, Handayaningsih AE, Rachman MP, Oktriani R, Kurniasari I. 2022. Wolbachia genetic similarity in different insect host species: *Drosophila melanogaster* and Yogyakarta's (Indonesia) *Aedes aegypti* as a novel host. Biodiversitas 23 (5). 2321-2328. DOI: 10.13057/biodiv/d230510.

Roy N, Lazzaretti C, Paradiso E, Capponi C, Ferrari T, Reggianini F, Sperduti S, Baschieri L, Mascolo E, Perri C. 2023. Short-term exposure to bisphenol A does not impact gonadal cell steroidogenesis in vitro. Cells. 12 (11): 1537. DOI: 10.3390/cells12111537.

Schober P, Boer C, Schwarte LA. 2018. Correlation coefficients: Appropriate use and interpretation. Anesth Analg 126 (5): 1763-1768. DOI: 10.1213/ANE.0000000000002864.

Sharma P, Sharma K, Sharma G, Chadha P. 2021 Dec 22. A review on the occurrence, exposure, and health impacts of bisphenol A. Toxicol Intl 28 (5): 337-356. DOI: 10.18311/ti/2021/v28i4/27473.

Smorodinskaya S, Kochetkov N, Gavrilin K, Nikiforov-Nikishin D, Reznikova D, Vatlin A, Klimuk A, Odorskaya M, Nikiforov-Nikishin A, Ponomarev A. 2023. The effects of acute bisphenol A toxicity on the hematological parameters, hematopoiesis, and kidney histology of Zebrafish (*Danio rerio*). Animals 13 (23): 3685. DOI: 10.3390/ani13233685.

Sorensen RM, Savić-Zdravković D, Jovanović B. 2024. Changes in the wing shape and size in fruit flies exposed to micro and nanoplastics. Chemosphere 363: 142821. DOI: 10.1016/j.chemosphere.2024.142821.

Sun L, Mu Y, Xu L, Han X, Gu W, Zhang M. 2023. Transgenerational inheritance of wing development defects in *Drosophila melanogaster* induced by cadmium. Ecotoxicol Environ Saf 250: 114486. DOI: 10.1016/j.ecoenv.2022.114486.

Szabla N, Maria LA, Antoł A, Sobczyk Ł, Angilletta MJ, Czarnoleski M. 2024. Evolution and development of *Drosophila melanogaster* under different thermal conditions affected cell sizes and sensitivity to paralyzing hypoxia. J Insect Physiol 157: 104671. DOI: 10.1016/j.jinsphys.2024.104671.

Tepe Y, Çebi A. 2019. Acrylamide in environmental water: A review on sources, exposure, and public health risks. Expo Heal 11 (1): 3-12. DOI: 10.1007/s12403-017-0261-y.

Tripathy NK, Patnaik KK, Nabi MJ. 1991. Acrylamide is genotoxic to the somatic and germ cells of *Drosophila melanogaster*. Mutat Res Toxicol 259 (1): 21-27. DOI: 10.1016/0165-1218 (91)90105-U.

Tsai W-T. 2023. Survey on the environmental risks of bisphenol A and its relevant regulations in Taiwan: An environmental endocrine-disrupting chemical of increasing concern. Toxics 11 (9): 722. DOI: 10.3390/toxics11090722.

Vigneron A, Geffard O, Quéau H, François A, Chaumot A. 2019. Nongenetic inheritance of increased Cd tolerance in a field *Gammarus fossarum* population: Parental exposure steers offspring sensitivity. Aquat Toxicol 209: 91-98. DOI: 10.1016/j.aquatox.2019.02.001.

Volz SN, Poulsen R, Hansen M, Holbech H. 2024. Bisphenol A alters retinal morphology, visually guided behavior, and thyroid hormone levels in zebrafish larvae. Chemosphere 348: 140776. DOI: 10.1016/j.chemosphere.2023.140776.

Wang J, Chan FKS, Johnson MF, Chan HK, Cui Y, Chen J, Chen W-Q. 2025. Material cycles, environmental emissions, and ecological risks of bisphenol A (BPA) in China and implications for sustainable plastic management. Environ Sci Technol 59 (3): 1631-1646. DOI: 10.1021/acs.est.4c09876.

Wu Y, Li Y, Jia W, Zhu L, Wan X, Gao S, Zhang Y. 2023. Reconstructing hepatic metabolic profile and glutathione-mediated metabolic fate of acrylamide. Environ Pollut 337: 122508. DOI: 10.1016/j.envpol.2023.122508.

Yu WZ, Shen P, Lim I, Shi RRS, Cai M, Chin YS, Tay AJ, Ang WM, Er JC, Lim GS. 2023. Occurrence and dietary exposure to acrylamide from foods consumed within and outside main meals in Singapore. Foods 12 (16): 3022. DOI: 10.3390/foods12163022.

Zahrah NA, Ramadhan MJ, Kharomah S, Kharomah S, Choirunisa N, Zubaidah S, Fauzi A. 2025. Wing damage and size reduction in *Drosophila melanogaster* caused by bisphenol A. BIO Web Conf 183: 01007. DOI: 10.1051/bioconf/202518301007.