INTEGRATED IN SILICO AND IN VIVO APPROACH TO DECIPHER REPRODUCTIVE TOXICITY OF PLASTIC MONOMERS IN DROSOPHILA MELANOGASTER

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Introduction:

The widespread use of plastics in modern industries has led to increasing concerns about the environmental and biological impact of their monomeric components. Plastic monomers such as bisphenol-A, styrene, and acrylonitrile are known to leach into the environment, posing potential risks to living organisms. Among these risks, reproductive toxicity has emerged as a critical area of investigation due to its long-term implications on population health and ecosystem stability. However, comprehensive studies linking molecular interactions to organism-level reproductive effects remain limited.

This project aims to address this gap by integrating *in silico* and *in vivo* approaches to evaluate the reproductive toxicity of selected plastic monomers. The model organism *Drosophila melanogaster* is widely used due to its well-characterized genetics, short life cycle, and sensitivity to environmental changes. Initially, computational methods such as ADMET prediction and molecular docking will be employed to identify high-risk monomers and their interactions with key fertility-related proteins. Subsequently, experimental validation will be conducted through controlled feeding trials to determine toxicity thresholds, including LD_{50} and sublethal doses. The study will also assess the internal uptake of monomers and their effects on reproductive parameters such as fecundity, embryonic viability, and mating efficiency. By correlating computational predictions with biological outcomes, this research provides a comprehensive framework for understanding plastic-induced reproductive toxicity.

Overall, the study contributes to environmental toxicology by generating valuable data that can support safer chemical design, regulatory policies, and public health awareness regarding plastic exposure.

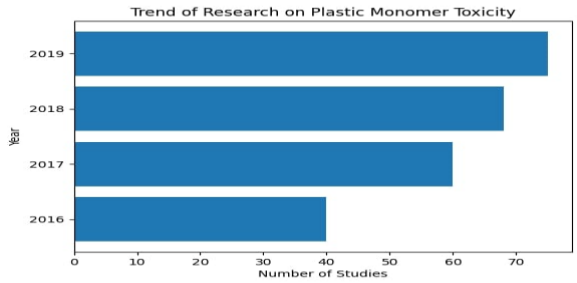


Figure 1: Trend of Research on Plastic Monomer-Induced Reproductive Toxicity (2016–2019)

Objectives:

1. Retrieval and ADMET Prediction of Plastic Monomers. (Achieved)
2. Molecular Docking and Interaction Analysis of Plastic Monomers with Fertility Related Key Targets of *Drosophila melanogaster*. (Achieved)
3. Determination of Lethal Dose (LD50) and Sublethal Dose of Selected Plastic Monomers. (Yet to Achieve)
4. Assessment of Plastic Monomers Uptake. (Yet to Achieve)
5. Determination of Reproductive Toxicity of Plastic Monomers in *Drosophila melanogaster.* (Yet to Achieve)
6. Publications (One Review and One Research Article). (Yet to Achieve)

Methodology:

1. Retrieval and ADMET prediction of plastic monomers

   The selected plastic monomers; Acrylic acid (6581), Adipic acid (196), Caprolactam (7768), Ethylene (6325), Ethylene glycol (174), Propylene (8252), Vinyl chloride (6338), Hexamethylenediamine (16402), Styrene (7501), and Terephthalic acid (7489) was retrieved from the PubChem database in SDF format based on their global industrial prevalence and environmental relevance (Kim et al., 2019). Ligand preparation was performed using Open Babel and PyRx (Dallakyan & Olson, 2015), involving structure cleaning, energy minimization, hydrogen addition, and conversion into PDBQT format at physiological pH. ADMET characteristics of each monomer was predicted using SwissADME (Daina et al., 2017) and ProTox-II (Banerjee et al., 2018) to evaluate physicochemical properties, pharmacokinetics, and toxicity. Comparative analysis of these ADMET profiles will enable the identification of high risk monomers, which will subsequently be prioritized for docking and *in vivo* reproductive toxicity assessment in *Drosophila melanogaster*.

2. Molecular docking and interaction analysis of plastic monomers with fertility related key targets of *Drosophila melanogaster.*

   The key fertility related proteins in *Drosophila melanogaster*; Ecdysone Receptor, Ovary specific Kinesin, Vasa, Testis specific Kinesin, and Dynein was retrieved from the PDB database (McGrail & Hays, 2002; Januschke & Gonzalez, 2010; Lu et al., 2022). These proteins regulate oocyte maturation, intracellular cargo transport, germ cell specification, spermatid elongation, and sperm motility. Protein structures was prepared using Discovery Studio Visualizer by removing water molecules, adding hydrogens, correcting missing residues, and performing energy minimization. The

optimized structures is converted to PDBQT format and used in PyRx for molecular docking studies with selected plastic monomers, enabling analysis of potential interactions and fertility related effects.

Result and Conclusion:

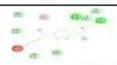
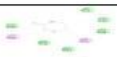
The ADMET analysis of selected plastic monomers revealed considerable variation in their physicochemical and toxicity profiles. Compounds such as ethylene, propylene, and styrene exhibited low TPSA and higher LogP values, indicating good membrane permeability, whereas terephthalic acid, adipic acid, and hexamethylenediamine showed higher polarity and greater potential for protein interactions. All monomers followed Lipinski's rule of five, suggesting acceptable bioavailability, and none demonstrated blood-brain barrier permeability, indicating limited central nervous system exposure. Toxicity evaluation showed that acrylic acid and adipic acid had low LD_{50} values, indicating higher toxicity, while styrene and vinyl chloride exhibited moderate toxicity and ethylene glycol showed relatively low toxicity. Most compounds were categorized under toxicity classes 3–5, reflecting varying degrees of harmful effects. Overall, these findings suggest that plastic monomers possess the potential to interact with biological targets and may act as reproductive toxicants, highlighting the need for further molecular docking and in vivo validation studies.

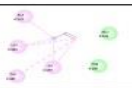
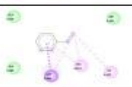
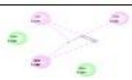
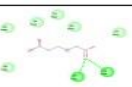
Table-1: ADMET profile of selected plastic monomers

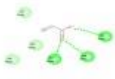
Molecules	Molecular formula	Molecular weight	Accession Number	Rotatable bonds	H- acceptor	H-Donor	TPSA	Consensus Log p	Solubility	GI absorption	BBB permeability	Pgp Substrate	CYP Inhibitions	Lipinski violation	Bioavailability score	Pain	Break	synthetic accessibility	Toxicity	Toxicity Class	LD50(mg/ kg)
Ethylene	C_2H_4	28.05g/mol	6325	0	0	0	0.00Å²	1.00	Very soluble	Low	No	No	No	0	0.55	0	1	1.90	Yes	4	1910mg/kg
Propylene	C_3H_6	42.08 g/mol	8252	0	0	0	0.00Å²	1.35	Very soluble	Low	No	No	No	0	0.55	0	1	1.24	Yes	5	3210mg/kg
Styrene	C_8H_8	104.15g/mol	7501	1	0	0	0.00 Å²	2.72	soluble	Low	No	No	No	0	0.55	0	0	1.00	Yes	4	316mg/kg
Terephthalic acid	$C_8H_6O_4$	166.13g/mol	7489	2	4	2	74.60Å²	1.13	Very soluble	High	No	No	No	0	0.85	0	0	1.00	Yes	5	2340mg/kg
Ethylene glycol	$C_2H_6O_2$	62.07 g/mol	174	1	2	2	40.46Å²	-0.70	Highly soluble	High	No	No	No	0	0.55	0	0	1.00	Yes	5	4700mg/kg
Vinyl chloride	C_2H_3Cl	62.50 g/mol	6338	0	0	0	0.00 Å²	1.27	Very soluble	Low	No	No	No	0	0.55	0	0	1.95	Yes	4	500mg/kg
Hexamethylenediamine	$C_6H_{16}N_2$	116.20 g/mol	16402	5	2	2	52.04Å²	0.56	Very soluble	High	No	No	No	0	0.55	0	0	1.00	Yes	4	380mg/kg
Adipic acid	$C_6H_{10}O_4$	146.14 g/mol	196	5	4	2	74.60Å²	0.38	Very soluble	High	No	No	No	0	0.85	0	0	1.17	Yes	3	94mg/kg
Caprolactam	$C_6H_{11}NO$	113.16 g/mol	7768	0	1	1	29.10Å²	0.74	Very soluble	High	No	No	No	0	0.55	0	0	1.01	Yes	4	560mg/kg
Acrylic acid	$C_3H_4O_2$	72.06 g/mol	6581	1	2	1	37.30Å²	0.23	Very soluble	High	No	No	No	0	0.85	0	1	1.07	Yes	2	34mg/kg

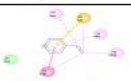
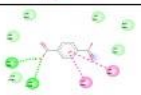
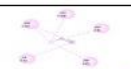
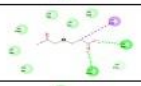
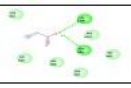
Among all plastic monomers, Terephthalic acid showed the strongest binding across all proteins. It exhibited highest affinity with kinesin (-5.4), dynein (-5.5), vasa (-4.8), and Ecdysone receptor (-4.7). Styrene also showed relatively strong binding, especially with kinesin (-5.4) and dynein (-4.9). Moderate binding was observed with Adipic acid and Caprolactam. Weak interactions were seen for Ethylene and Propylene. Hydrogen bond interactions were mainly observed with amino acids like Arg, Asn, Gly, and Leu. Terephthalic acid is the most potent ligand, indicating higher potential to disrupt protein function. Strong binding with motor proteins (kinesin, dynein) suggests possible interference in intracellular transport. Interaction with vasa protein indicates potential effects on germ cell development. Binding to ecdysone receptor suggests possible endocrine disruption in Drosophila.

Table-2: Binding affinities and key hydrogen bonding of plastic monomers with drosophila melanogaster target receptors

Receptors	Plastic monomers (ligand)	Binding affinity	Conventional hydrogen bond	2D interaction with plastic monomers
Ecdysone receptor	Ethylene	-1.5	-	
	Propylene	-1.5	-	
	Styrene	-4.0	-	
	Terephthalic acid	-4.7	Lys-66, Cys-49	
	Ethylene glycol	-2.9	Arg-33, Arg-63, Gly-26	
	Vinyl chloride	-1.9	-	
	Hexamethylenediamine	-3.1	Arg-47, Asn-48, Cys-49	
	Adipic acid	-4.0	Arg-33, Arg-56, Arg-63, Asn-57	
	Caprolactam	-4.0	-	
	Acrylic acid	-3.0	Gln-60	

Vasa protein	Ethylene	-1.7	-	
	Propylene	-2.3	-	
	Styrene	-4.2	-	
	Terephthalic acid	-4.8	Asn-225	
	Ethylene glycol	-2.7	Ile-156, Tyr-153	
	Vinyl chloride	-2.1	-	
	Hexamethylenediamine	-3.1	Leu-191	
	Adipic acid	-4.2	Arg-161, Ser-158	
	Caprolactam	-3.8	Arg-161, Ser-158	

	Acrylic acid	-3.0	Arg-161, Asn-225, Ser-158	
Kinesin	Ethylene	-1.9	-	
	Propylene	-2.6	-	
	Styrene	-5.4	-	
	Terephthalic acid	-5.4	Leu-75	
	Ethylene glycol	-3.1	Gly-78, Leu-75, Ser-220	
	Vinyl chloride	-2.3	-	
	Hexamethylenediamine	-3.8	Glu-15, Phe-54	
	Adipic acid	-4.8	Glu-15, Phe-54, Pro-55	
	Caprolactam	-5.0	Thr-310	
	Acrylic acid	-3.8	Asn-250	

Dynein	Ethylene	-1.8	-	
	Propylene	-2.5	-	
	Styrene	-4.9	-	
	Terephthalic acid	-5.5	Gly-89, Leu-109	
	Ethylene glycol	-2.8	Asn-30, Asn-97, Thr-99	
	Vinyl chloride	-2.1	-	
	Hexamethylenediamine	-3.4	Tyr-61	
	Adipic acid	-4.3	Gly-89, Leu-109	
	Caprolactam	-4.4	Gly-89, Leu-109, Thr-87	
	Acrylic acid	-3.5	Gly-89, Leu-109	

References:

1. [1] A. Daina, O. Michielin, and V. Zoete, “SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules,” Scientific Reports, vol. 7, no. 1, pp. 1–13, 2017.
2. [2] P. Banerjee, A. O. Eckert, A. K. Schrey, and R. Preissner, “ProTox-II: A webserver for the prediction of toxicity of chemicals,” Nucleic Acids Research, vol. 46, no. W1, pp. W257–W263, 2018**.**
3. [3] T. Nishimura et al., “Spectrophotometric methods for chemical uptake studies,” Journal of Analytical Science, vol. 28, no. 3, pp. 233–240, 2012**.**

Future Scope:

The future scope of this project includes:

1. Extension of the study to include more plastic monomers and their combined toxicity effects.
2. Validation of results in higher model organisms to assess relevance to human health.
3. Development of safer alternatives and predictive screening tools for plastic toxicity.