Integrative Review of Mitochondrial Genes in Zebrafish Cardio hepatic Toxicity Induced by Microplastics, Nanoparticles and Xenobiotics

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Abstract
Aquatic ecosystems are subject to escalating contamination by microplastics (MPs), engineered nanoparticles (NPs) and environmental xenobiotics yet the subcellular mechanisms through which these diverse contaminants converge to impair vertebrate organ function remain incompletely defined. This integrative review systematically examines the role of mitochondrial gene networks in mediating cardiohepatic toxicity in zebrafish (*Danio rerio*) exposed to MPs, NPs and xenobiotics drawing on evidence from transcriptomics, proteomics, metabolomics and functional mitochondrial biology. We demonstrate that across structurally diverse toxicant classes, a conserved molecular signature emerges comprising suppression of the PGC-1α/NRF1/TFAM biogenesis axis, transcriptional repression of both nuclear and mitochondrially encoded electron transport chain (ETC) subunits, dysregulation of mitochondrial fusion and fission dynamics, oxidative stress induced mtDNA damage and activation of the intrinsic apoptotic cascade. Toxicant class specific mechanisms metal ion mediated enzyme inhibition for metallic NPs, physical membrane intercalation for polymeric MPs and NPs, and CYP mediated bioactivation for xenobiotics which overlay these shared pathways generating mechanistically informative fingerprints for hazard characterization. The concurrent vulnerability of zebrafish cardiomyocytes and hepatocytes to mitochondrial dysfunction reflects their shared dependence on oxidative phosphorylation, high mitochondrial density and limited anaerobic capacity establishing the mechanistic basis of cardiohepatic toxicity crosstalk. Multi omics integration in zebrafish further reveals epigenetic propagation of mitochondrial dysfunction through CpG hypermethylation and histone remodelling at key biogenesis loci with potential transgenerational consequences. Validated mitochondrial biomarker panels including mtDNA copy number, ETC complex activities, ΔΨm and transcriptomic pathway scores which offer regulatory compatible endpoints for environmental risk assessment. These findings position zebrafish mitochondrial toxicogenomics as a powerful and translationally relevant platform for elucidating environment genome interactions underlying cardiovascular and hepatic disease in both aquatic biota and human populations.

Keywords: zebrafish, mitochondrial toxicogenomics, cardiohepatic toxicity, microplastics, nanoparticles, xenobiotics, oxidative phosphorylation, PGC-1α, mtDNA, environmental toxicology.

1. Introduction

1.1 Overview of Environmental Pollutants and Aquatic Contamination

The unprecedented expansion of industrialisation, urbanisation and agricultural intensification over the past century has precipitated a profound and escalating crisis of environmental contamination. Aquatic ecosystems rivers, lakes, estuaries and marine environments now serve as the terminal repositories for an extraordinarily diverse mixture of anthropogenic pollutants ranging from persistent organic compounds and heavy metals to emerging contaminants such as synthetic polymers and engineered nanomaterials [1]. These ecosystems which harbor nearly half of all known vertebrate species and play a critical role in the sustaining global food resources which are continuously exposed to the multiple chemical stressors that can act individually or interactively placing significant pressure on the adaptive resilience of aquatic organisms.

Freshwater bodies receive contamination from the multiple convergent pathways direct industrial effluents agricultural runoff laden with pesticides and fertilisers improperly treated municipal wastewater or by atmospheric deposition and leaching from landfill sites and plastic debris. Marine environments are further impacted by the transoceanic transport of persistent pollutants various deep sea mining activities and the fragmentation of plastic waste across the water column. The resulting chemical milieu is characterised by extraordinary complexity, temporal variability and spatial collection rendering straightforward dose response assessments both methodologically challenging and ecologically insufficient [2].

Of particular concern is the phenomenon of bioaccumulation whereby lipophilic or protein binding contaminants concentrate progressively through the successive trophic levels of aquatic food webs a process termed biomagnification. Organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and more recently detected perfluoroalkyl and polyfluoroalkyl substances (PFAS) exemplify this pattern achieving tissue concentrations in apex predators many orders of the magnitude above ambient environmental levels [3]. The implications for wildlife populations and for human consumers of aquatic organisms are correspondingly severe.

Critically current environmental monitoring frameworks and regulatory thresholds frequently fail to account for the mixture toxicity the interactive effects that arise when organisms are simultaneously exposed to multiple contaminants. Additive, synergistic and antagonistic interactions among coinciding pollutants can dramatically

alter the toxicological outcomes predicted from the single compound studies. A comprehensive mechanistic understanding of how specific pollutant classes perturb fundamental biological systems particularly at the subcellular and molecular level is therefore an indispensable prerequisite for the development of ecologically meaningful risk assessment models and protective environmental policies [4].

1.2 Definition and Significance of Microplastics, Nanoparticles and Xenobiotics

Among the most significant categories of contemporary aquatic contaminants are microplastics, engineered nanoparticles and the broader class of xenobiotics each presenting distinct physicochemical properties, environmental behaviours and biological interaction profiles that collectively necessitate integrative toxicological scrutiny.

Microplastics are operationally defined as plastic particles measuring less than 5 millimetres in their longest dimension encompassing a heterogeneous assemblage of polymeric materials that differ in composition (polyethylene, polypropylene, polystyrene, polyethylene terephthalate and others), shape (fragments, fibres, pellets, films, beads), surface chemistry and degree of weathering [5]. Primary microplastics are manufactured at small size for industrial or cosmetic applications including microbeads in personal care products and pre-production nurdles used as raw polymer feedstock. Secondary microplastics arise through the progressive physical, photochemical and biological fragmentation of larger plastic items under environmental stress. The ubiquity of microplastic contamination has been demonstrated across virtually every environmental compartment investigated to date including remote polar ice cores, deep-sea sediments, cloud water and the tissues of diverse aquatic organisms. Their ecological significance extends beyond physical obstruction and pseudo-satiation weathered microplastic surfaces acquire complex eco corona layers of adsorbed organic molecules, metals and microbial biofilms that substantially modify their bioavailability and toxicological behaviour, and which may facilitate the co-transport of sorbed contaminants including persistent organic pollutants and antimicrobials into biological tissues in a so called Trojan horse mechanism [6].

Nanoparticles, conventionally defined as particles with at least one dimension in the 1–100 nanometre range encompass an equally diverse array of materials including titanium dioxide (TiO_2), zinc oxide (ZnO), silver (Ag), gold (Au), cerium dioxide (CeO_2), carbon-based nanomaterials (fullerenes, carbon nanotubes, graphene oxide) and quantum dots. Their extraordinary surface area to volume ratio, quantum imprisonment effects and the enhanced chemical reactivity confer properties qualitatively distinct from those of bulk materials of equivalent composition. These characteristics underlie their broad commercial utility in applications spanning medicine, electronics, cosmetics, textiles, agriculture and environmental remediation. However the same attributes that render nanoparticles technologically attractive also govern their biological interactions in different ways that conventional ecotoxicological frameworks are ill equipped to accommodate. Nanoparticles can translocate across epithelial and endothelial barriers accumulate within the organelles including mitochondria and the nucleus generate reactive oxygen species (ROS) through photocatalysis or Fenton type reactions and dysregulate signalling cascades in a manner largely independent of ionic dissolution [7].

The term xenobiotic (from the Greek xenos, meaning foreign and bios, meaning life) encompasses any chemical substance that is foreign to the biological system under consideration, regardless of origin. In the context of aquatic toxicology xenobiotics most commonly refer to synthetic organic compounds that enter aquatic environments through human activity including pharmaceuticals and their active metabolites endocrine disrupting compounds (EDCs) such as bisphenol A (BPA), phthalates and synthetic estrogen polycyclic aromatic hydrocarbons, dioxins and furans, organochlorine and organophosphate pesticides and industrial solvents. Many xenobiotics are characterised by structural similarity to endogenous biological molecules, enabling them to interact with receptors, enzymes and transcription factors in ways that mimic, block or amplify normal biological signals often at extraordinarily low concentrations. Their capacity to disrupt endocrine homeostasis, immune function and developmental programming in aquatic vertebrates including fish has been documented with increasing precision in both field and laboratory settings [8].

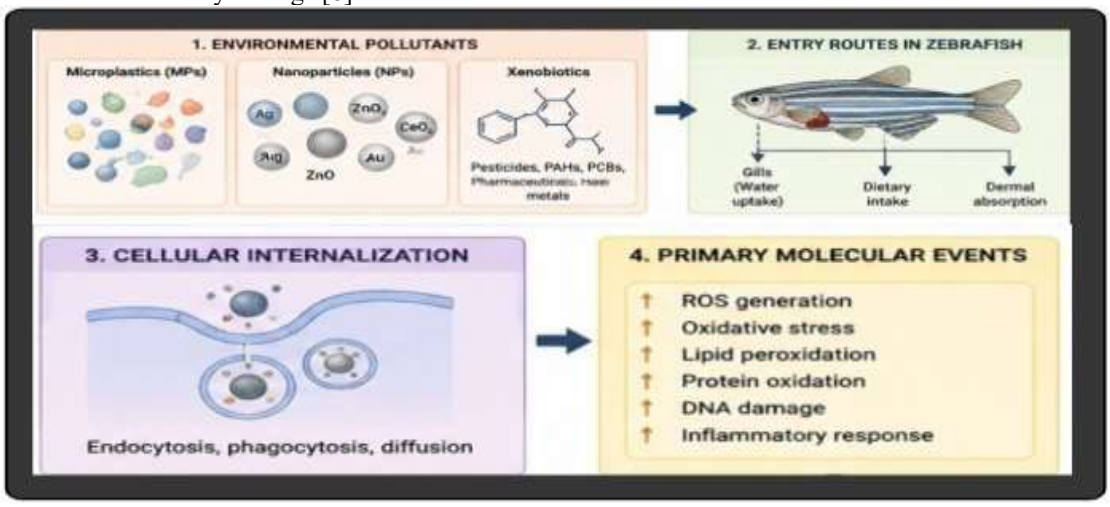

Figure 1. Mechanistic Overview of Pollutant Entry, Cellular Internalization and Oxidative Stress Induction in Zebrafish

1.3 Importance of Mitochondria in Cellular Homeostasis

Mitochondria are essential double membrane organelles of the endosymbiotic origin that occupy a position of unparalleled centrality in the regulation of cellular life and death. Their canonical function the synthesis of adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS) underpins virtually every energy demanding process in the eukaryotic cells from active ion transport and macromolecular biosynthesis to cytoskeletal dynamics and signal transduction [9]. However, the modern mitochondrial biology has revealed a breadth of additional functions that positions these organelles as master integrators of cellular homeostasis far beyond their classical bioenergetic role.

The mitochondrial electron transport chain (ETC) comprising complexes I through IV embedded in the inner mitochondrial membrane couples the stepwise oxidation of NADH and $FADH_2$ to the vectorial pumping of the protons across the inner membrane establishing the electrochemical gradient (mitochondrial membrane potential, $\delta\Psi m$) that drives ATP synthase (complex V). This process is inherently associated with the generation of reactive oxygen species primarily superoxide anion as a byproduct of electron leakage at complexes I and III. Under physiological conditions, endogenous antioxidant systems including manganese superoxide dismutase (MnSOD/SOD2), glutathione peroxidase (GPx) and thioredoxin reductase maintain ROS within a range compatible with redox signalling functions [10]. However, when ETC function is compromised by toxicant exposure mitochondrial ROS production escalates dramatically precipitating oxidative damage to mitochondrial DNA (mtDNA) or membrane phospholipids and respiratory chain proteins in a self amplifying spiral of mitochondrial dysfunction.

Beyond bioenergetics, mitochondria occupy nodal positions in the regulation of intrinsic apoptosis, calcium buffering, steroidogenesis, haem biosynthesis, iron-sulphur cluster assembly and the integration of nutrient sensing pathways. Mitochondria associated membranes (MAMs) physical contact sites between the outer mitochondrial membrane and the endoplasmic reticulum coordinate calcium transfer, lipid biosynthesis and autophagosome formation in ways that critically influence cellular stress responses. The mitochondrial permeability transition pore (mPTP) a supramolecular channel that opens in response to elevated matrix calcium, oxidative stress or membrane potential dissipation represents a critical decision point between reversible injury and irreversible cell death serving as the effector mechanism for both necrotic and apoptotic cell death programmes in hepatocytes and cardiomyocytes [11].

The mitochondrial genome (mtDNA) in vertebrates is a circular, double stranded DNA molecule of approximately 16 - 17 kilobases encoding 13 essential OXPHOS subunits (ND1-6, ND4L, COX1-3, ATP6, ATP8, Cytb), 22 transfer RNAs and 2 ribosomal RNAs. Unlike the nuclear genome mtDNA exists in multiple copies per cell (polyploidy) which lacks protective histone packaging that possesses limited DNA repair capacity and is positioned in immediate proximity to the primary intracellular source of ROS rendering it exceptionally vulnerable to oxidative mutagenesis. The mitochondrial transcription factor A (TFAM) and associated regulatory proteins (TFB1M, TFB2M, POLRMT) govern mtDNA replication and transcription, while the PGC-1α/NRF1/NRF2 axis coordinates nuclear-encoded mitochondrial biogenesis in response to energetic and oxidative signals. Disruption of nuclear mitochondrial communication by environmental toxicants can therefore propagate dysfunction from a localised mitochondrial insult into a systemic impairment of cellular bioenergetics and stress resilience [12].

Mitochondria are particularly susceptible to the environmental contaminants due to several intrinsic structural and functional characteristics. The negative membrane potential of the mitochondrial matrix promotes the accumulation of lipophilic cationic compounds while the extensive inner mitochondrial membrane provides a large surface area for the interaction and incorporation of amphipathic toxicants. Furthermore, mitochondria are the primary intracellular source of reactive oxygen species (ROS) making them especially vulnerable to oxidative stress induced by environmental pollutants. Unlike nuclear DNA, mitochondrial DNA (mtDNA) possesses limited repair mechanisms and lacks protective histone proteins which increasing its susceptibility to damage and mutation.. Mitochondrial dysfunction therefore represents not merely one among many toxic endpoints but a fundamental mechanistic hub through which diverse classes of environmental contaminants including microplastics, nanoparticles and xenobiotics converge to disrupt cellular homeostasis [13].

1.4 Concept of Cardiohepatic Toxicity

Cardiohepatic toxicity refers to the concurrent mechanistically linked impairment of cardiac and hepatic function resulting from xenobiotic exposure. Although traditionally regarded as distinct organ systems within separate subspecialties of clinical medicine and toxicology. The heart and liver are functionally and anatomically interconnected through multiple bidirectional axes haemodynamic, metabolic, inflammatory and humoral that render them mutually vulnerable to shared pathological insults.

The anatomical intimacy of the cardiohepatic relationship is exemplified by the portal and hepatic venous circulations. The liver receives approximately 75% of its blood supply from the portal vein which drains the intestinal microvasculature and thus constitutes the primary duct through which ingested or intestinally absorbed toxicants reach the liver at high concentrations before systemic dilution. The remaining 25% of hepatic perfusion derives from the hepatic artery itself a branch of the coeliac trunk arising from the aorta. Hepatic venous outflow returns to the right heart via the inferior vena cava and subsequently traverses in the pulmonary circulation before coronary perfusion [13], [14]. This circulatory architecture means that toxicants absorbed from the gastrointestinal tract are delivered to the liver at maximal concentrations and that hepatically processed metabolites which may be more or less toxic than the parent compound are subsequently delivered to the coronary circulation. In zebrafish larvae, the analogous hepatoportal circulation is established by 5 dpf and similarly positions the liver as the primary metabolic gatekeeper for intestinally absorbed contaminants [15].

At the cellular level, both hepatocytes and cardiomyocytes share several features that contribute to their co-susceptibility to mitochondrial toxicants. Both cell types are characterised by exceptionally high metabolic rates, an almost exclusive dependence on oxidative phosphorylation for ATP generation (particularly in the case of cardiomyocytes which obtain approximately 95% of their energy from OXPHOS) and correspondingly high mitochondrial density in cardiomyocytes, mitochondria occupy approximately 30-35% of total cell volume [16]. Both cell types express high levels of fatty acid oxidation enzymes that are critically dependent on mitochondrial function and are sensitive to disruption of the carnitine shuttle. Both are poorly equipped with anaerobic glycolytic capacity and therefore poorly tolerant of OXPHOS inhibition. And both express the mPTP components cyclophilin D, VDAC and ANT that render them susceptible to permeability transition mediated cell death under conditions of oxidative and energetic stress.

Emerging clinical and experimental evidence documents a syndrome of mutual organ injury: cardiotoxic compounds that reduce cardiac output secondarily impair hepatic perfusion causing ischaemic hepatocellular injury and impairing the metabolic processing of additional toxicants in a self amplifying cycle. Conversely, hepatotoxic agents that impair liver function reduce the biotransformation and elimination of cardiotoxic compounds, prolong their systemic half-lives and may generate reactive metabolites that reach the coronary circulation in increased concentrations [17]. This bidirectional amplification of toxicological injury which may be termed cardiohepatic toxicity crosstalk is particularly relevant to environmental contaminants such as microplastics, nanoparticles and xenobiotics which are typically encountered as complex mixtures and which exert mechanistic effects at the mitochondrial level in both target organs simultaneously.

In zebrafish, the spatial proximity of the developing heart and liver during organogenesis combined with their shared dependence on yolk-derived nutrients metabolised via mitochondrial β-oxidation which creates a developmental window of exceptional co-vulnerability. The mechanistic basis for this syndrome and in particular the role of specific mitochondrial genes in mediating or modifying it forms the central subject of the present review [18].

1.6 Aim and Objectives of the Review

The primary aim of this integrative review is to synthesise, critically evaluate and conceptually organise the existing body of evidence pertaining to the role of mitochondrial gene expression and function in mediating cardiohepatic toxicity in zebrafish exposed to microplastics, nanoparticles and xenobiotics. By drawing together findings from comparative toxicogenomic, developmental biology, environmental chemistry and mitochondrial physiology. This review seeks to identify convergent mechanistic pathways, reconcile contradictions in the literature, highlight critical knowledge gaps and propose a unified conceptual framework for future research and regulatory application.

The specific objectives of this review are as follows:

- To provide a comprehensive and critically annotated overview of the classes of environmental contaminants microplastics, nanoparticles and xenobiotics most commonly implicated in aquatic toxicology, with particular attention to their physicochemical properties, environmental behaviour and mechanisms of biological uptake in zebrafish.
- To review the structure, function and toxicological vulnerability of the zebrafish mitochondrial proteome and genome with emphasis on those genes and gene products whose expression is most consistently altered in response to environmental contaminant exposure.
- To systematically evaluate published transcriptomic, proteomic and functional studies documenting alterations in mitochondrial gene expression and activity in zebrafish cardiac tissue following exposure to each major pollutant class.
- To perform an equivalent evaluation of mitochondrial gene responses in zebrafish hepatic tissue with attention to the specific metabolic functions of the liver and the role of mitochondrial β-oxidation, OXPHOS and ROS homeostasis therein.
- To identify and critically analyse the molecular mechanisms through which disrupted mitochondrial gene function in the heart and liver are interrelated including shared upstream regulators, common metabolic vulnerabilities and inter organ signalling mechanisms so as to construct a mechanistically coherent framework of cardiohepatic toxicity.

This review is structured to provide a logical and progressive exposition moving from the characterisation of pollutant classes and the establishment of the zebrafish model's mechanistic context through organ-specific analyses of mitochondrial responses, toward an integrative synthesis of cardiohepatic mechanisms and translational implications. It is hoped that the resulting framework will serve as a valuable resource for environmental toxicologists, mitochondrial biologists, regulatory scientists and aquatic ecologists working at the interface of molecular biology and environmental health.

2. Mitochondrial Biology and Genetic Organization

2.1 Structure and Function of Mitochondria

Mitochondria are highly dynamic, double-membrane-bound organelles that serve as the principal bioenergetic engines and metabolic hubs of eukaryotic cells. Derived from an ancient endosymbiotic event involving an alphaproteobacterial ancestor approximately 1.5 to 2 billion years ago, mitochondria have retained a residual genome encoding a small but indispensable set of core respiratory chain subunits, while the vast majority of mitochondrial proteins estimated at approximately 1,100-1,500 in vertebrates are encoded by the nuclear genome,

synthesised in the cytosol and imported via specialised translocase complexes (TOM and TIM complexes) into the appropriate mitochondrial compartment [19]. The result is an organelle of extraordinary structural sophistication and functional versatility whose integrity is a prerequisite for cellular viability in virtually all aerobic eukaryotes.

Structurally, mitochondria are delimited by two concentric membranes that define three distinct compartments: the outer mitochondrial membrane (OMM), the intermembrane space (IMS) and the inner mitochondrial membrane (IMM) which encloses the mitochondrial matrix. The OMM is relatively permeable to small molecules (up to approximately 5 kDa) via voltage-dependent anion channels (VDAC/porin) but maintains a selective barrier to larger proteins. The IMM is highly impermeable to ions and polar molecules a property essential for the maintenance of the proton electrochemical gradient and is elaborated into extensive, finger like invaginations termed cristae which dramatically amplify the inner membrane surface area and accommodate the densely packed respiratory chain complexes and ATP synthase [20]. The geometry of cristae is not static cristae remodelling, governed by the dynamin like GTPase OPA1 and the MINOS/MICOS complex critically influences the efficiency of OXPHOS by controlling the local concentration of protons within the cristae lumen.

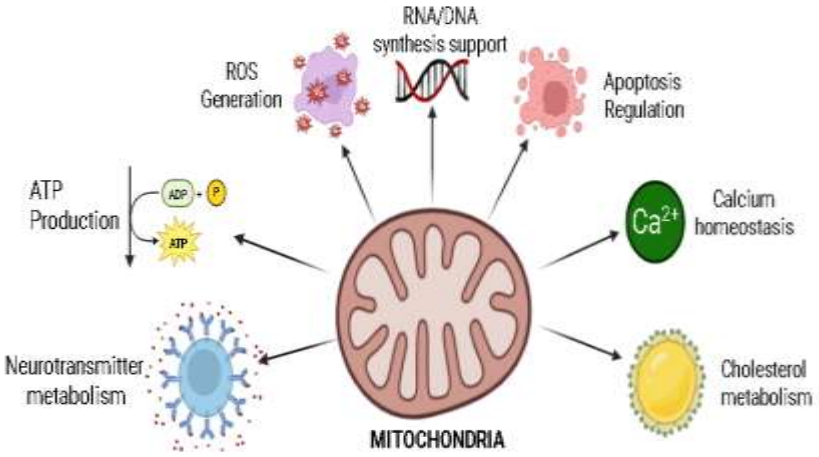

Figure 2. Various Functions of Mitochondria

2.1.1 ATP Production

The synthesis of adenosine triphosphate from ADP and inorganic phosphate by mitochondrial ATP synthase (complex V is also designated F_1F_0-ATPase) represents the quantitatively most important pathway of ATP generation in aerobic eukaryotes under normal physiological conditions. Complex V harnesses the energy stored in the proton electrochemical gradient composed of both an electrical component ($\Delta\Psi$, the membrane potential, approximately - 150 to -180 mV) and a chemical component (ΔpH, approximately 0.5 to 1.0 units alkaline in matrix) to drive the condensation of ADP and Pi through a rotary catalytic mechanism [21]. The F_0 sector, embedded in the IMM comprises a ring of c-subunits whose rotation is driven by proton translocation this rotation is transmitted via the central stalk to the catalytic β-subunits of the F_1 sector in the matrix, inducing conformational changes that sequentially bind ADP and Pi, catalyse ATP synthesis and release the product. The stoichiometry of proton translocation per ATP synthesised is approximately 3.67 H^+/ATP in mammals meaning that the P/O ratio (ATP molecules per oxygen atom reduced) approaches theoretical values of approximately 2.5 for NADH oxidation and 1.5 for $FADH_2$ [22].

The efficiency of ATP synthesis is critically dependent on IMM integrity as any proton leak pathway whether physiological (via uncoupling proteins such as UCP2 and UCP3) or pathological (induced by lipid peroxidation products, detergents or membrane intercalating toxicants) dissipates the proton gradient as heat rather than driving ATP synthesis [23]. This uncoupling phenomenon is a well-documented mechanism of mitochondrial toxicity for multiple environmental contaminants including certain polycyclic aromatic hydrocarbons, substituted phenols and fatty acid like compounds and is of particular relevance to the cardiotoxic and hepatotoxic effects of xenobiotics in zebrafish.

2.1.2 Oxidative Phosphorylation

Oxidative phosphorylation is the integrated process by which the stepwise transfer of electrons from reduced coenzymes (NADH, $FADH_2$) to molecular oxygen is coupled to the phosphorylation of ADP to ATP via the proton electrochemical gradient. The process is executed by five multimeric protein complexes embedded in the IMM: complex I (NADH: ubiquinone oxidoreductase), complex II (succinate: ubiquinone oxidoreductase), complex III (ubiquinol: cytochrome c oxidoreductase), complex IV (cytochrome c oxidase) and complex V (ATP synthase) collectively constituting the respiratory chain [24], [25].

Complex I, the largest of the respiratory chain complexes (approximately 980 kDa in mammals, comprising 44 subunits of which 7 are mtDNA-encoded), oxidises NADH, reduces ubiquinone (coenzyme Q) to ubiquinol and translocates four protons across the IMM per electron pair. Complex II, the only OXPHOS complex entirely encoded by nuclear DNA, oxidises succinate to fumarate and transfers electrons to ubiquinone without contributing to the proton gradient. Complex III (cytochrome bc_1 complex) oxidises ubiquinol, reduces cytochrome c and translocates four protons per electron pair via the Q-cycle mechanism. Complex IV (cytochrome

c oxidase) accepts electrons from reduced cytochrome c, reduces molecular oxygen to water and translocates two protons per electron pair. Recent structural and functional evidence from cryo-electron tomography has revealed that these complexes are not randomly distributed within the IMM but are organised into higher order supramolecular assemblies termed respirasomes or super complexes principally $I_1III_2IV_1$ that may enhance electron transfer efficiency, reduce ROS generation and stabilise individual complex subunits [26].
In zebrafish, the OXPHOS system is functionally and genetically conserved relative to mammals with orthologues identified for all 44 subunits of complex I, the four subunits of complex II, the 11 core subunits of complex III and the 14 core subunits of complex IV. The developmental expression of OXPHOS subunits in zebrafish follows a precisely regulated temporal programme with marked upregulation accompanying the maternal to zygotic transition (approximately 3-4 hpf) and the onset of organogenesis reflecting the shifting bioenergetic requirements from glycolysis dependent early cleavage stages to OXPHOS dependent differentiated tissues [27].

2.1.3 Reactive Oxygen Species (ROS) Generation
Reactive oxygen species are generated as inevitable byproducts of mitochondrial electron transport arising primarily from the univalent reduction of molecular oxygen at complexes I and III. At complex I, the flavin mononucleotide (FMN) cofactor and several iron sulphur clusters are capable of directly reducing O_2 to superoxide anion ($O_2\bullet^-$), particularly when the NADH/NAD^+ ratio is elevated (reverse electron transport (RET)) or when the ubiquinone pool becomes highly reduced. At complex III, the ubisemiquinone radical intermediate generated during the Q-cycle can donate an electron to O_2 on both the matrix and IMS faces of the IMM. Additional mitochondrial ROS sources include the pyruvate dehydrogenase complex, α-ketoglutarate dehydrogenase and the electron transferring flavoprotein (ETF)/ETF-ubiquinone oxidoreductase system involved in fatty acid β-oxidation [28].
Superoxide is rapidly dismutated to hydrogen peroxide (H_2O_2) by manganese superoxide dismutase (SOD2/MnSOD) in the matrix and copper-zinc SOD (SOD1/CuZnSOD) in the IMS. H_2O_2 is itself a relatively stable, membrane permeant oxidant that can serve as a second messenger in redox signalling when present at low concentrations but which undergoes Fenton or Haber-Weiss reactions with transition metal ions (particularly Fe^{2+} and Cu^+) to generate the highly reactive hydroxyl radical (•OH) the most potent biological oxidant known. Hydroxyl radicals react non selectively with virtually all biological macromolecules at diffusion limited rates, causing single strand breaks in mtDNA, initiating lipid peroxidation chain reactions in membrane phospholipids (generating 4-hydroxynonenal and malondialdehyde as reactive electrophilic products) and oxidising cysteine, methionine and other redox-sensitive amino acid residues in proteins [29].
The mitochondrial antioxidant network comprising SOD2, glutathione peroxidase 1 (GPx1) and GPx4 (phospholipid hydroperoxide GPx), peroxiredoxins 3 and 5 (Prx3, Prx5), thioredoxin 2 (Trx2), thioredoxin reductase 2 (TrxR2) and glutaredoxin 2 (Grx2) normally maintains matrix ROS at levels compatible with physiological redox signalling while preventing oxidative damage. Environmental toxicants can disrupt this balance through multiple mechanisms: by inhibiting specific ETC complexes and increasing electron leakage by depleting antioxidant cofactors (particularly glutathione), by directly inhibiting antioxidant enzymes or by generating additional ROS through redox cycling (as occurs with paraquat, diquat and certain quinone metabolites). In zebrafish exposed to microplastics, nanoparticles and xenobiotics, ROS overproduction and oxidative stress markers including 8-hydroxy-2'-deoxyguanosine (8-OHdG) in mtDNA, protein carbonyls and lipid peroxidation products are among the most consistently reported mechanistic endpoints [30].

2.2 Mitochondrial Genome and Key Genes
The mitochondrial genome (mtDNA) of vertebrates represents one of the most compact and efficiently organised genetic systems known in biology encoding an essential subset of the organelle's protein complement alongside the RNA machinery required for its own translation. Understanding the organisation, expression and vulnerability of the zebrafish mitochondrial genome is fundamental to interpreting the transcriptional responses observed following environmental toxicant exposure [31].
2.2.1 mtDNA Organization in Zebrafish
The zebrafish mitochondrial genome is a circular and double stranded DNA molecule of 16,596 base pairs seen first fully sequenced in 1998. Like mammalian mtDNA it encodes 13 protein coding genes (all OXPHOS subunits), 22 transfer RNA (tRNA) genes and 2 ribosomal RNA (rRNA) genes (12S and 16S rRNA). The two strands are designated the heavy (H) strand and the light (L) strand based on their buoyant density in caesium chloride gradients reflecting differences in guanine and thymine content. The H-strand encodes 12 of the 13 protein coding genes both rRNAs and 14 of the 22 tRNAs, the L-strand encodes only the ND6 subunit and the remaining 8 tRNAs [32].
A non-coding control region of approximately 1 kilobase the displacement loop (D-loop) contains the origins of H-strand replication (OH) and the promoters for H-strand and L-strand transcription (HSP and LSP respectively). The D-loop is the most variable region of the mitochondrial genome and houses conserved sequence blocks (CSBs I, II and III) that are essential for the formation of the RNA primers required for H-strand replication initiation. Zebrafish mtDNA is present in thousands of copies per cell in metabolically active tissues such as the heart and liver with copy number itself constituting a regulated parameter of mitochondrial biogenesis [33].
Zebrafish mtDNA lacks introns and contains minimal intergenic sequences in several instances, adjacent genes overlap by a few nucleotides and stop codons for some genes are completed post-transcriptionally by polyadenylation of the mRNA. Replication proceeds via a strand displacement mechanism initiating at OH with the origin of L-strand replication (OL) situated approximately two thirds around the genome within a tRNA gene

cluster [34]. Transcription is initiated at HSP and LSP producing polycistronic precursor transcripts that are subsequently processed by cleavage at the tRNA punctuation signals flanking each gene.

Table 1. Protein coding genes of the zebrafish mitochondrial genome

Gene	Complex	Subunit Function	Strand	Key Role in Toxicology	References
ND1	I	Core proton-pumping subunit	H	Inhibited by rotenone; mutation elevates ROS	[35]
ND2	I	Core subunit, NADH binding domain	H	Altered in heavy metal & microplastic exposure	[36]
ND3	I	Accessory structural subunit	H	Marker of mitochondrial dysfunction	[37]
ND4	I	Core proton-translocation subunit	H	Mutation associated with optic neuropathy models	[38]
ND4L	I	Accessory subunit ND4 interaction	H	Expression suppressed by PAH exposure	[39]
ND5	I	Largest core subunit; proton pump	H	Frequently downregulated in xenobiotic studies	[40]
ND6	I	Core subunit; unique L-strand gene	L	Hypermethylation reported under oxidative stress	[41]
COX1	IV	Catalytic subunit; O_2 reduction site	H	Key biomarker; altered by nanoparticle exposure	[42]
COX2	IV	Cytochrome c oxidation site	H	Reduced in BPA and phthalate exposures	[43]
COX3	IV	Structural; proton channel regulation	H	Dysregulated in polystyrene microplastic studies	[44]
CYTB	III	Ubiquinol oxidation & Q-cycle	H	Used as phylogenetic marker; ROS sensor	[45]
ATP6	V	Proton channel of F_0 sector	H	Expression correlates with $\Delta\Psi m$ disruption	[46]
ATP8	V	Assembly/stabilisation of F_0	H	Smallest mtDNA gene; early stress indicator	[36]

Table 2. Key nuclear-encoded mitochondrial genes in zebrafish toxicology

Gene	Protein	Mitochondrial Role	Toxicological Relevance	References
TFAM	Mitochondrial transcription factor A	mtDNA packaging, replication initiation, transcription	Master regulator; suppressed by ROS, nanoparticles, xenobiotics	[37]
POLG	DNA polymerase gamma	mtDNA replication and repair	Inhibited by NRTI drugs; affected by arsenite and cadmium	[47]
PGC-1α (ppargc1a)	PGC-1α	Master regulator of mitochondrial biogenesis	Suppressed by microplastics, EDCs, and chronic oxidative stress	[48]
NRF1	Nuclear respiratory factor 1	Transcriptional activator of OXPHOS & TFAM	Co-regulated with PGC-1α; biomarker of biogenesis impairment	[49]
SOD2	Mn-superoxide dismutase	Matrix ROS scavenging ($O_2^{\bullet-} \rightarrow H_2O_2$)	Induced early; suppressed in chronic or severe toxicant exposure	[50]
VDAC1	Voltage-dependent anion channel 1	OMM permeability; metabolite exchange; mPTP component	Altered by BPA, heavy metals; mPTP sensitisation	[51]
DRP1 (dnm1l)	Dynamin-related protein 1	Mitochondrial fission GTPase	Elevated by nanoparticles and microplastics; promotes fragmentation	[52]

Gene	Protein	Mitochondrial Role	Toxicological Relevance	References
MFN2	Mitofusin 2	OMM fusion; ER-mitochondria contacts	Suppressed in toxicant-induced fragmentation phenotypes	[53]
OPA1	Optic atrophy protein 1	IMM fusion; cristae remodelling	Cleavage by OMA1 activated by ΔΨm loss; ROS-sensitive	[54]
PINK1	PTEN-induced kinase 1	Mitophagy initiation; kinase on depolarised mitochondria	Upregulated in early toxicant response; mitophagy flux indicator	[55]
PRKN (parkin)	Parkin (E3 ubiquitin ligase)	Selective autophagy receptor for damaged mitochondria	Co-upregulated with PINK1; zebrafish parkin KO models used	[56]
HSP60 (hspd1)	Heat shock protein 60	Matrix chaperone; mtUPR component	Induced by misfolded protein accumulation; xenobiotic stress	[57]

2.3 Mitochondrial Dynamics

Mitochondria are not static organelles but exist in a perpetual state of morphological flux undergoing continuous cycles of fusion and fission, targeted degradation via selective autophagy (mitophagy) and de novo biogenesis. These dynamical processes are mechanistically interrelated and collectively serve to maintain a functional mitochondrial network that is appropriately matched to cellular energetic demands, capable of responding adaptively to physiological stimuli and competent to segregate and eliminate damaged components before they impair the function of the broader mitochondrial population [58]. The dysregulation of mitochondrial dynamics is increasingly recognised as a fundamental mechanism of toxicant induced injury in zebrafish cardiac and hepatic tissues.

2.3.1 Fusion and Fission

Mitochondrial fusion the merging of adjacent mitochondrial outer and inner membranes to form a single continuous organelle is executed by a set of dynamin-related GTPases that act sequentially on the two membranes. OMM fusion is mediated by Mitofusin 1 (MFN1) and Mitofusin 2 (MFN2) transmembrane GTPases that form trans-oligomeric complexes between adjacent mitochondria and use GTP hydrolysis to drive membrane tethering and fusion [59]. IMM fusion is subsequently executed by OPA1 (Optic Atrophy Protein 1) a multimeric GTPase anchored in the IMM that also governs cristae architecture, its activity requires prior OMM fusion and is regulated by proteolytic processing: long isoforms (L-OPA1) are essential for fusion while short isoforms (S-OPA1) generated by stress activated proteases (OMA1, YME1L) cannot support fusion and when L-OPA1 is depleted promote cristae opening and cytochrome c release. Mitochondrial fusion promotes the mixing and complementation of mtDNA, proteins and metabolites between organelle diluting locally damaged components and maintaining a homogeneous and functional mitochondrial network so it is therefore particularly important under conditions of partial mitochondrial dysfunction [60].

Mitochondrial fission the division of a single mitochondrion into two daughter organelles is principally executed by Dynamin-Related Protein 1 (DRP1, encoded by DNM1L in zebrafish) a cytosolic GTPase that is recruited to the OMM by adaptor proteins including MiD49, MiD51, MFF and FIS1, oligomerises into ring like structures at pre-determined constriction sites (often coinciding with ER-mitochondria contact sites) and uses GTP hydrolysis to mechanically constrict and sever the mitochondrial membranes. Fission serves physiologically to facilitate mitochondrial distribution during cell division enable mitophagy by producing small, segregated units that can be engulfed by autophagosomes and release damaged membrane segments from the network. However, excessive or unregulated fission characterised by mitochondrial fragmentation into small, rounded, disconnected units is a hallmark of toxicant-induced mitochondrial injury and is associated with reduced OXPHOS capacity, membrane potential dissipation and initiation of apoptotic cascades through the release of pro-apoptotic intermembrane space proteins (cytochrome c, SMAC/Diablo, AIF, Endo G) [61].

In zebrafish, genetic knockdown of mfn1, mfn2 or opa1 produces cardiac defects including pericardial oedema, reduced stroke volume and arrhythmia while dnm1l knockdown causes hepatomegaly and biliary dysgenesis directly establishing the requirement for balanced fusion-fission dynamics in cardiohepatic development. Environmental toxicants including polystyrene microplastics, zinc oxide nanoparticles and bisphenol A have been shown to shift the fusion-fission balance toward fission in zebrafish larvae evidenced by increased DRP1 recruitment to mitochondria, reduced MFN2 and OPA1 protein levels and the appearance of fragmented mitochondrial networks in live imaging studies using fluorescent mitochondrial reporter lines such as Tg(mito-Kaede) and Tg(mito-EGFP) [62].

2.3.2 Mitophagy

Mitophagy is the selective autophagic degradation of damaged, depolarised or superfluous mitochondria within lysosomes representing the principal mechanism for mitochondrial quality control at the organelle level. The best

characterised mitophagy pathway in vertebrates is the PINK1-Parkin axis when mitochondrial membrane potential ($\Delta\Psi m$) is dissipated, PINK1 (PTEN-induced kinase 1) accumulates on the OMM (rather than being imported and proteolytically cleaved under basal conditions) where it phosphorylates ubiquitin and the ubiquitin ligase Parkin at Serine 65 activating Parkin's E3 ligase activity. Activated Parkin ubiquitinates multiple OMM proteins (including VDAC1, MFN1, MFN2 and Miro) generating polyubiquitin chains that recruit autophagy receptors (NDP52, OPTN, p62/SQSTM1) and the autophagy initiation machinery ultimately resulting in the engulfment of the flagged mitochondrion by a phagophore membrane and its delivery to the lysosome for degradation [63]. Additional mitophagy receptors that operate in a ubiquitin-independent manner including BNIP3, NIX/BNIP3L and FUNDC1 are activated under hypoxic conditions or by specific post-translational modifications and contribute to mitophagy in zebrafish cardiac and hepatic tissues under physiological and toxicant induced stress.
Mitophagy flux the dynamic rate of mitochondrial delivery to and degradation within lysosomes can be assessed in zebrafish using fluorescent reporter systems that exploit the pH sensitivity of fluorescent proteins: mito-Keima (a pH-sensitive fluorescent protein that shifts emission from green to red in the acidic lysosomal environment) and tandem fluorescent reporter constructs (mito-mCherry-GFP, in which GFP fluorescence is quenched in the lysosome while mCherry persists) enable real-time non-invasive monitoring of mitophagic activity in living zebrafish embryos. Studies employing these tools have demonstrated impaired mitophagy flux in zebrafish exposed to polystyrene nanoplastics and silver nanoparticles with accumulation of depolarised, ubiquitin-positive mitochondria and suppression of PINK1, Parkin signalling contributing to the progressive accumulation of dysfunctional mitochondria in cardiac and hepatic tissues [64].
An important distinction must be drawn between adaptive mitophagy a protective response that efficiently removes damaged mitochondria before they can initiate cell death cascades and excessive mitophagy that depletes the mitochondrial network beyond the capacity of biogenesis to replace it ultimately resulting in energetic failure and cell death. Both failure of mitophagy initiation (due to inadequate PINK1 or Parkin activation) and excessive mitophagy (due to overwhelming mitochondrial damage) can contribute to zebrafish cardiohepatic toxicity and the net outcome is critically dependent on the intensity, duration and mechanism of the toxicant insult [65].

2.3.3 Mitochondrial Biogenesis

Mitochondrial biogenesis is the process by which cells expand their mitochondrial mass and mtDNA copy number in response to increased energetic demand, exercise, caloric restriction or recovery from mitochondrial injury. The master transcriptional co-activator PGC-1α (peroxisome proliferator-activated receptor gamma co-activator 1-alpha encoded by PPARGC1A) is the central positive regulator of this process, integrating upstream signals from AMPK (activated by low cellular ATP/AMP ratio), SIRT1 (the NAD^+-dependent deacetylase activated under conditions of metabolic stress) and CaMKIV (activated by Ca^{2+} signalling) to co-activate Nuclear Respiratory Factors 1 and 2 (NRF1, NRF2) which in turn drive the expression of TFAM (the primary regulator of mtDNA replication and transcription) and nuclear encoded OXPHOS subunits co-ordinately expanding both the mtDNA content and the respiratory capacity of the cell [66].
In zebrafish, ppargc1a is expressed throughout the embryo from early somitogenesis stages with highest expression in the developing heart, liver and skeletal muscle reflecting the bioenergetic intensity of these tissues. Morpholino mediated knockdown of ppargc1a in zebrafish produces a phenotypic syndrome closely resembling the cardiohepatic toxicity observed following environmental contaminant exposure: reduced heart rate, pericardial oedema, hepatomegaly and reduced mtDNA copy number, underscoring the central role of mitochondrial biogenesis in the maintenance of cardiohepatic function. Environmental toxicants suppress ppargc1a expression through multiple converging mechanisms: excessive ROS production activates NF-κB signalling and inflammatory cytokine release that transcriptionally suppress PGC-1α, epigenetic modifications including CpG hypermethylation at the PGC-1α promoter have been documented following exposure to bisphenol A and cadmium and disruption of AMPK-SIRT1 signalling by energy depleting toxicants removes the upstream activating input to PGC-1α [67], [68].
The interplay between mitophagy and biogenesis is of particular relevance to the interpretation of toxicant-induced cardiohepatic dysfunction in zebrafish. Under conditions of moderate, acute toxicant challenge both processes may be simultaneously upregulated mitophagy removing damaged organelles and biogenesis replacing them resulting in mitochondrial network renewal without net loss of capacity. Under conditions of chronic or severe toxicant exposure. However the biogenic response may be overwhelmed or directly suppressed leading to progressive net mitochondrial loss, energetic failure and the cascade of cardiohepatic pathology described in subsequent sections of this review. The balance point between adaptive renewal and pathological depletion is a central determinant of the toxicological outcome and represents an important target for the future research and biomarker development [69].

3. Zebrafish as a Model for Cardiohepatic Toxicity

3.1 Advantages of the Zebrafish Model

The selection of an appropriate model organism for an investigating mechanism of environmental toxicity is a decision of fundamental methodological consequence directly determining the biological depth, translational validity, experimental throughput and ultimate regulatory applicability of the research programme. The zebrafish has achieved its current preeminence in the environmental toxicology not through the predominance of any single advantage but through the unique convergence of genetic tractability, developmental accessibility, physiological conservation and practical utility that collectively position it as the most powerful vertebrate system currently available for mechanistic cardiohepatic toxicity research [70].

3.1.1 Genetic Similarity with Humans

The zebrafish genome, sequenced and assembled through a collaborative international effort culminating in the Genome Reference Consortium Zebrafish Build 11 (GRCz11) comprises approximately 1.4 gigabases distributed across 25 chromosome pairs and encodes an estimated 26,200 protein coding genes. Comparative genomic analyses have established that approximately 70% of human protein coding genes have at least one zebrafish orthologue and that this figure rises to over 82% when restricted to human disease genes catalogued in the Online Mendelian Inheritance in Man (OMIM) database. The conservation is not merely at the level of sequence identity but extends to syntenic organisation, regulatory element function, developmental expression timing and protein-protein interaction networks properties that collectively support the translational validity of zebrafish mechanistic findings for human biology [71].

Of particular relevance to mitochondrial toxicology the zebrafish mitochondrial proteome is highly conserved relative to the human orthologue. All 13 mtDNA encoded proteins share sequence identity of 50-75% with their human counterparts, with functional conservation that has been directly validated by heterologous complementation experiments. The nuclear encoded components of the OXPHOS complexes, mitochondrial import machinery, biogenesis regulators (PGC-1α, NRF1, TFAM) and dynamics effectors (MFN1/2, OPA1, DRP1, PINK1, Parkin) are similarly well conserved typically sharing greater than 60% amino acid identity with human proteins. Crucially, the principal xenobiotic metabolising enzymes CYP1A (the principal PAH metabolising cytochrome P450 in fish), CYP3A, CYP2K and others and the nuclear receptors that govern their transcription (AhR, PXR, CAR, ERα, ERβ, TRα/β) are structurally and functionally conserved in zebrafish enabling toxicokinetic and toxicodynamic studies with direct relevance to human xenobiotic exposure [72].

A further dimension of genetic utility is the availability of an extensive array of zebrafish mutant and transgenic resources. The Zebrafish Information Network (ZFIN) catalogues over 35,000 mutant alleles and more than 5,000 transgenic lines representing a genetic toolkit of unmatched scope among vertebrate model organisms [73]. For mitochondrial toxicology specifically established zebrafish mutant lines lacking functional pink1, prkn, opa1, mfn2, tfam and polg have provided invaluable in the vivo platforms for dissecting the genetic requirements for mitochondrial quality control in the cardiac and hepatic development and for testing whether specific toxicant effects are mediated through these pathways.

3.1.2 Transparent Embryos

Perhaps the most operationally distinctive feature of zebrafish embryos and early larvae is their optical transparency which enables direct non-invasive visualisation of internal organ development and physiology at single cell resolution in a living vertebrate. This transparency arises from the virtual absence of melanin pigmentation in the skin, the small tissue thickness of embryos and larvae (less than 1 mm) and the high refractive index uniformity of early stage tissues. Standard laboratory strains such as AB, Tubingen and WIK maintain transparency through approximately 24-48 hpf before melanophore differentiation begins; the Casper double mutant ($roy^{-/-}$; $nacre^{-/-}$) which lacks both melanophores and iridophores remains transparent throughout all life stages enabling adult organ imaging [74].

The practical implications for toxicology are transformative. Zebrafish embryos and larvae can be loaded into multicell plates and imaged by automated high content microscopy systems to simultaneously quantify heart rate, cardiac output, stroke volume, pericardial area (a sensitive indicator of cardiac oedema), liver area and morphology, yolk sac absorption, blood flow velocity and vascular integrity all in unanaesthetised living animals at throughputs of hundreds to thousands of specimens per day [75]. Fluorescent transgenic reporter lines add molecular resolution to these morphological assessments: mitochondrial membrane potential can be monitored in real time using JC-1 or TMRM dye accumulation; ROS production can be quantified using MitoSOX or genetic H_2O_2 sensors such as HyPer and mt-HyPer cell death can be tracked through Tg(caspase3:DEVD-GFP) or AO/PI vital staining and specific gene transcription can be visualised in situ using fluorescent in situ hybridisation (FISH) or live smFISH in reporter lines [76].

Multiphoton and light sheet fluorescence microscopy (LSFM) have further expanded the optical capabilities of zebrafish as a toxicological model enabling volumetric imaging of intact organs at subcellular resolution with minimal phototoxicity. Three dimensional reconstructions of the developing heart and liver combined with correlated fluorescent reporter data and computational quantification have produced mechanistic insights into toxicant induced cardiomyopathy and hepatocellular injury that would be entirely inaccessible in the opaque mammalian embryos or in vitro cell culture systems [77].

3.1.3 Rapid Organ Development

Zebrafish embryos and larvae develop with extraordinary rapidity completing the formation of functional cardiac, hepatic, renal, neural and the haematopoietic systems within five days of fertilisation at 28.5°C. The heart begins rhythmic contraction at approximately 22 hpf and achieves mature two chamber morphology with the functional valves and a complete circulatory loop by 72 hpf. The liver is specified by 16 hpf which visible as a distinct organ bud by 32 hpf and achieves functional competence for xenobiotic metabolism act as a glycogen storage, lipid processing and bile production by 5 dpf. This temporal compression of organogenesis into a five-day developmental window creates an extraordinarily efficient experimental timeline that is logistically, economically and ethically superior to equivalent mammalian developmental studies [78].

From a regulatory perspective, zebrafish embryos and early larvae (prior to 5 dpf) are not classified as protected animals under many international regulatory frameworks including European Union Directive 2010/63/EU (which regulates the use of animals for scientific purposes) enabling their use in large-scale screening studies with reduced ethical constraints relative to mammalian models. This regulatory classification, combined with the low cost of zebrafish husbandry, the high fecundity of adult pairs and the small size of embryos that permits exposure in

multicell plate formats using microgram quantities of test compound collectively enable experimental designs of population level statistical power that are unachievable in mammalian systems [79].
The rapidity of zebrafish development also facilitates the study of sensitive windows of developmental vulnerability discrete temporal intervals during which specific organ systems are undergoing rapid morphogenesis and are therefore disproportionately susceptible to chemical disruption. For the heart and liver, the periods of maximal organogenetic sensitivity (approximately 24–72 hpf and 32–96 hpf, respectively) can be precisely targeted by stage specific toxicant exposures, enabling the dissection of developmental stage specific mechanisms of cardiohepatic toxicity and the identification of critical windows for risk assessment purpose [80].

3.2 Zebrafish Heart and Liver Physiology

3.2.1 Cardiac Structure and Function

The zebrafish heart is a two-chambered structure comprising a single atrium and a single ventricle, separated by an atrioventricular (AV) canal that houses bicuspid valve leaflets and connected to the aortic outflow tract via the bulbus arteriosus (an elastic, muscular structure functionally analogous to the mammalian cardiac valves and proximal aorta). Despite the apparent simplicity of this two chamber plan relative to the four chamber mammalian heart, the molecular and cellular basis of zebrafish carcinogenesis is fundamentally conserved with mammals, with orthologous transcription factors (Nkx2.5, GATA4, Tbx5, Hand1/2), signalling pathways (Nodal/Bone morphogenetic protein, FGF, Notch, Wnt) and structural proteins (cardiac troponins, myosin's, actins, connexins) governing each stage of heart formation [81].
The zebrafish ventricular myocardium is organised as a bilaminar structure: an outer, compact cortical layer (the equivalent of the mammalian compact myocardium) and an inner, spongy trabeculated layer (the equivalent of the mammalian trabecular myocardium and ventricular septum precursor). Trabeculation the formation of muscular ridges projecting into the ventricular lumen is driven by Neuregulin-1/ErbB2/ErbB4 signalling and Notch pathway activation and is essential for ventricular compliance, oxygen and nutrient delivery to the inner myocardium and ultimately cardiac output. Toxicant induced impairment of trabeculation in zebrafish, manifested as reduced trabecular density and ventricular compaction defects, is a morphological phenotype of particular mechanistic interest as it recapitulates human left ventricular non-compaction cardiomyopathy [82].
Cardiac electrophysiology in zebrafish is similarly conserved with mammals. The zebrafish heart rate (approximately 120-180 beats per minute at 28.5°C in larvae) is driven by spontaneous action potentials originating in a sinoatrial pacemaker region functionally analogous to the mammalian sinoatrial node, expressing HCN4 channels for the funny current (If) and exhibiting calcium clock-dependent automaticity [83]. Ventricular action potentials are characterised by a prominent plateau phase sustained by L-type calcium current (ICaL), rapid delayed rectifier potassium current (IKr, carried by the hERG/Kcnh2 channel that is also the principal target of drug induced QT prolongation in humans) and slow delayed rectifier potassium current (IKs, carried by KCNQ1/KCNE1). This electrophysiological profile renders zebrafish ventricular myocytes highly sensitive to compounds that block hERG channels or alter intracellular calcium homeostasis a sensitivity that is directly relevant to the arrhythmogenic effects of xenobiotics, nanoparticles and microplastic-adsorbed chemicals reported in zebrafish toxicology studies [84].

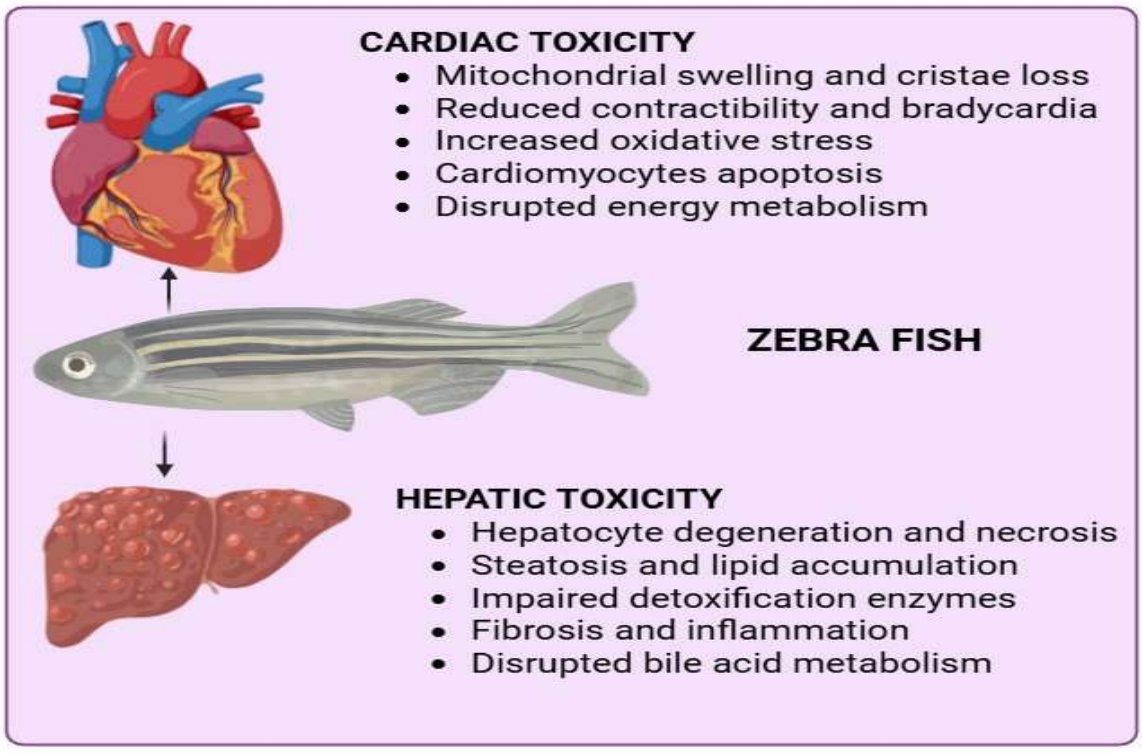

Figure 3. Cardiac and Hepatic Responses to Environmental Toxicants in Zebrafish

Mitochondria constitute approximately 30-35% of the total volume of mature zebrafish cardiomyocytes and are arranged in highly ordered arrays between the myofibrils a spatial organisation that facilitates ATP delivery to the actin-myosin cross bridge cycle and rapid calcium buffering near the sarcoplasmic reticulum. This organisation is recapitulated in adult zebrafish but is not fully established in embryonic and larval cardiomyocytes creating a developmental trajectory of increasing mitochondrial density and OXPHOS reliance that is precisely the period during which environmental toxicant exposures are most commonly applied in experimental studies. The vulnerability of this developmental window to mitochondrial toxicants is therefore not merely a consequence of immature detoxification capacity but also reflects the heightened energetic demand of a rapidly expanding increasingly OXPHOS dependent myocardium [85].

3.2.2 Hepatic Metabolism and Detoxification

The zebrafish liver develops from the endodermal hepatoblast progenitor population that is specified at the 10-somite stage (approximately 14 hpf) in the anterior endoderm by the combinatorial action of Wnt2bb, BMP2b, FGF10 and Notch signalling together with the transcription factors Prox1, Hhex and Tbx3. Hepatoblasts undergo proliferative expansion migration along the vitelline vein toward the developing gut tube and differentiation into hepatocytes (parenchymal cells) and biliary epithelial cells (cholangiocytes) in a process tightly regulated by Notch Jagged signalling. The mature zebrafish liver by 5 dpf is a compact highly vascularised organ exhibiting classical hepatocyte cord architecture, a biliary system draining into the gallbladder and intestinal lumen and a sinusoidal vasculature perfused by the hepatoportal circulation [86].

Xenobiotic metabolism in the zebrafish liver proceeds through phase I biotransformation (primarily oxidation by cytochrome P450 monooxygenases, expressed in the hepatocyte endoplasmic reticulum and mitochondria), phase II conjugation (via UDP-glucuronosyltransferases, sulfotransferases and glutathione-S-transferases) and phase III export (via ABC transporters including Mrp1/ABCC1, Mrp2/ABCC2 and P-glycoprotein/MDR1). The CYP1A enzyme (encoded by cyp1a in zebrafish) in the transcriptional target of the aryl hydrocarbon receptor (AhR) is the principal phase I enzyme responsible for the metabolism of planar polycyclic aromatic hydrocarbons, dioxins and certain microplastic adsorbed contaminants; its induction is used as a standard biomarker of PAH and dioxin like compound exposure in zebrafish toxicology studies using transgenic reporter lines such as Tg(CYP1A:GFP). Critically while phase I metabolism frequently increases the water solubility and excretability of xenobiotics so it can also generate reactive electrophilic and free radical intermediates (such as epoxides and quinones) that are more toxic than the parent compound and these metabolites may reach the mitochondria and directly impair OXPHOS function [87], [88].

Mitochondrial fatty acid β-oxidation (FAO) in zebrafish hepatocytes is the primary pathway for energy generation during fasting and yolk consumption in embryos and larvae and is also essential for the processing of fatty acid-containing xenobiotics and their metabolites. FAO is critically dependent on the mitochondrial carnitine shuttle (mediated by CPT1A and CPT2 on the outer and inner mitochondrial membranes respectively), the trifunctional protein (HADHA/HADHB) and the electron-transferring flavoprotein (ETF/ETFDH) system. Environmental toxicants that impair CPT1A activity including several phthalate metabolites and long-chain perfluoroalkyl substances or that inhibit the trifunctional protein as observed with valproic acid and certain aminoglycosides produce a characteristic hepatic phenotype of micro vesicular steatosis (hepatic lipid accumulation) in zebrafish reflecting the failure to oxidise accumulated fatty acids [89]. This steatotic phenotype is readily visualised in living zebrafish using Oil Red O staining, BODIPY fluorescent neutral lipid dyes or transgenic lipid reporter lines and represents a key cardiohepatic toxicity endpoint of direct clinical relevance given the epidemic of non-alcoholic fatty liver disease in human populations exposed to multiple environmental contaminants [90].

3.3 Toxicogenomic Applications in Zebrafish

Toxicogenomics the integrative study of how genomes respond to toxicant exposure, encompassing transcriptomics, proteomics, metabolomics, epigenomics and their integration has found in the zebrafish an exceptionally amenable experimental platform. The combination of genetic tractability, developmental accessibility, conservation of xenobiotic metabolising and signalling systems and the availability of genome wide molecular biology resources has positioned zebrafish toxicogenomics as a transformative approach for mechanism-based hazard characterisation, biomarker discovery and regulatory application [91].

4. Microplastics Induced Mitochondrial Toxicity

4.1 Sources and Characteristics of Microplastics

Microplastics (MPs) are heterogeneous contaminants defined as plastic particles <5 mm in diameter originating from the fragmentation of macro-plastic debris (secondary MPs) or manufactured at microscale for industrial and cosmetic applications (primary MPs). Dominant polymer types detected in the aquatic environments include polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyethylene terephthalate (PET) each exhibiting distinct physicochemical properties like density, hydrophobicity, surface charge and adsorption capacity that command their environmental fate and the biological interactions. Critically, MPs serve as vectors for co-contaminants which show adsorbing persistent organic pollutants (POPs), heavy metals and endocrine disrupting chemicals (EDCs) onto their hydrophobic surfaces thereby amplifying the toxic potential through combinatorial exposure [92]. Nanoplastics (NPs <1 μm) represent a physiochemically distinct subdivision which characterized by higher surface area to volume ratios that's increased the cellular penetrability and greater protein corona formation conferring disproportionate biological reactivity relative to their bulk counterparts.

4.2 Accumulation in Zebrafish Organs

The zebrafish (*Danio rerio*) model has proven instrumental in the deciphering organ specific bioaccumulation kinetics of MPs due to the optical transparency of larvae and the anatomical and physiological homology with mammalian systems. Fluorescently labelled PS microspheres administered at environmentally relevant concentrations (1-100 μg/L) undergo rapid gastrointestinal uptake within 24 hours of exposure with subsequent translocation to the liver, heart, gills and intestine confirmed by the confocal microscopy and flow cytometry. Hepatic accumulation is particularly pronounced consistent with the liver's central role in xenobiotic detoxification that ultrastructural analysis reveals MP deposition in hepatocyte cytoplasm or bile canaliculi and Kupffer cell phagolysosomes [93]. In the cardiac tissue, MPs concentrate within the pericardial space and the ventricular myocardium disrupting sarcomeres architecture. Particle size governs tissue distribution nanoscale fractions (<200 nm) penetrate mitochondrial membranes and nuclear envelopes whereas microscale fractions are predominantly confined to Endo lysosomal compartments which establishing a size dependent hierarchy of subcellular toxicity.

4.3 Effects on Mitochondrial Genes

Oxidative Stress Pathways Mitochondria represent a primary intracellular target of MP toxicity. PS-NP exposure activates the Nrf2/Keap1 signalling axis transiently inducing antioxidant gene expression before culminating in the oxidative overload, evidenced by elevated 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels and lipid peroxidation products. Dysregulation of *mt-nd1*, *mt-nd4* and *mt-cyb* genes encoding core NADH dehydrogenase and cytochrome b subunits mechanistically links MP exposure to the impaired electron handling and superoxide overproduction within Complex I and III [94].

Electron Transport Chain Disruption MPs physically intercalate with the inner mitochondrial membrane disrupting lipid bilayer integrity and the electrochemical proton gradient essential for the oxidative phosphorylation (OXPHOS). RNA sequencing studies in zebrafish exposed to PS-MPs identify significant transcriptional repression of *mt-co1*, *mt-co2*, *mt-atp6* and *mt-atp8* encoding cytochrome c oxidase and ATP synthase subunits of the Complex IV and V respectively. Corresponding enzymatic assays confirm dose dependent reductions in Complex I/III activity (>40% inhibition at 1 mg/L), mitochondrial membrane potential collapse (ΔΨm) and decreased the cellular ATP content. Metabolomic data further corroborate ETC dysfunction showing accumulation of succinate and NADH alongside depletion of citrate cycle intermediates consistent with a blockade at the ubiquinone reduction step [95].

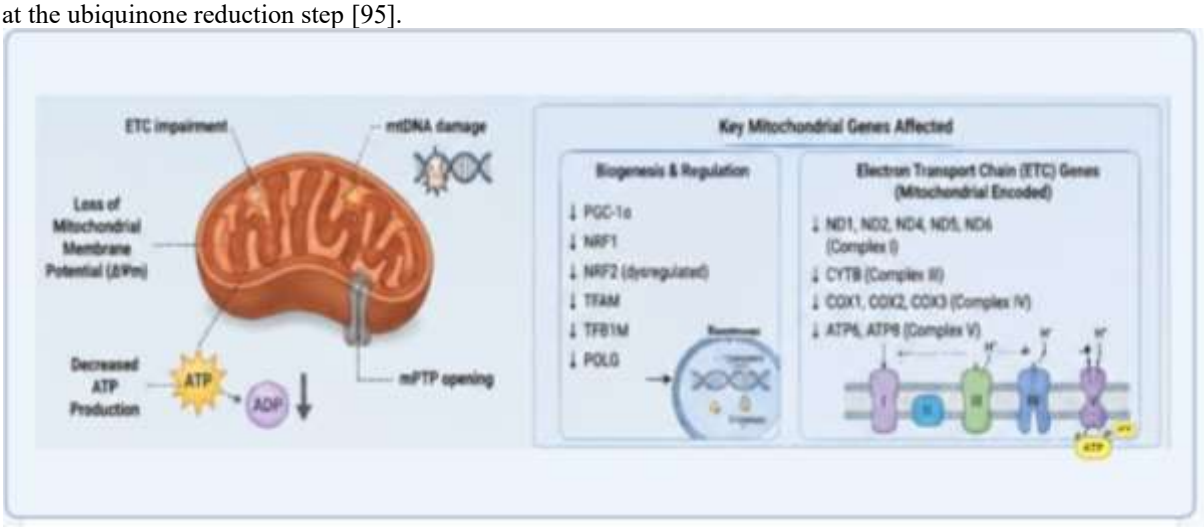

Figure 4. Schematic Representation of Mitochondrial Dysfunction and Differential Expression of Mitochondrial Genes

mtDNA Damage The mitochondrial genome is uniquely vulnerable to oxidative assault due to its proximity to ROS generating ETC complexes that show limited DNA repair capacity and the absence of protective histones. MP exposed zebrafish exhibit quantifiable mtDNA strand breaks detectable by long range PCR alongside elevated mitochondrial copy number in a compensatory biogenesis response to the genomic damage [96]. Mutations in the displacement loop (D-loop) control region which governs mtDNA replication and transcription have been documented following chronic PS-MP exposure with functional consequences including reduced mitochondrial transcription factor A (*tfam*) binding efficiency and suppressed expression of the entire mitochondrial gene complement. These findings establish mtDNA integrity as a sensitive sentinel biomarker of MP induced mitochondrial stress.

4.4 Cardiac Toxicity Mechanisms

Altered Heartbeat Quantitative analysis of cardiac function in the zebrafish larvae exposed to MPs demonstrates significant bradycardia, arrhythmia and reduced stroke volume at concentrations as low as 0.1 mg/L PS-MPs. High speed video microscopy reveals atrioventricular conduction delays and irregular ventricular filling patterns consistent with electrophysiological disturbance. Mechanistically microplastics induced mitochondrial dysfunction depresses ATP availability to the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) which impairing calcium cycling and the action potential repolarization. Transcriptional analysis identifies suppression of *cacna1c*

(L-type calcium channel), *ryr2* (ryanodine receptor 2) and *scn5lab* (cardiac sodium channel) in MP exposed hearts indicating convergent disruption of ionic homeostasis and excitation contraction coupling [97].

Mitochondrial Dysfunction in Cardiomyocytes Cardiomyocytes are among the most mitochondria dense cells in the vertebrate body (~30% of cell volume) rendering them acutely susceptible to the energetic failure. Transmission electron microscopy of MP exposed zebrafish hearts reveals mitochondrial swelling, cristae disorganization and outer membrane rupture morphological hallmarks of permeability transition pore (mPTP) opening. Transcriptomic data confirm upregulation of *ppif* (encoding cyclophilin D, the mPTP regulator), *bnip3* and *pink1* alongside suppression of fusion mediators *mfn1*, *mfn2* and *opa1* collectively indicating impaired mitochondrial quality control and a shift toward fragmented dysfunctional networks. The resulting bioenergetic collapse triggers cardiomyocyte apoptosis through mitochondria dependent caspase activation that show contributing to myocardial hypoplasia and reduced cardiac output observed in chronic MP exposure paradigms [98].

4.5 Hepatic Toxicity Mechanisms

Lipid Metabolism Disruption The liver is the principal organ of lipid homeostasis and MP induced mitochondrial dysfunction converges on the multiple nodes of hepatic lipid metabolism. Impaired β-oxidation consequent to reduced *cpt1a* and *acox1* expression and ETC mediated NAD^+ depletion which drives intrahepatic lipid accumulation detectable as Oil Red O positive lipid droplets in the zebrafish liver sections. Concurrently, aberrant activation of sterol regulatory element binding proteins (*srebp-1c*, *srebp-2*) upregulates *fasn* and *acc1* expression promoting de novo lipogenesis. This dual mechanism suppressed fatty acid oxidation combined with enhanced lipid synthesis phenocopies non alcoholic fatty liver disease (NAFLD) pathogenesis positioning the zebrafish MP model as a relevant platform for studying environment linked metabolic liver disease [99].

Hepatocellular Degeneration Histopathological analysis of MP exposed zebrafish livers reveals progressive hepatocellular degeneration characterized by nuclear pyknosis, cytoplasmic vacuolation, sinusoidal congestion and biliary hyperplasia. At the molecular level mitochondrial ROS activates hepatic stellate cells through the TGF-β1/Smad pathway initiating fibrogenic gene expression including *col1a1* and *acta2*. Simultaneously, NFE2L2 (Nrf2) pathway saturation permits sustained oxidative damage to hepatocellular proteins and lipids, triggering NLRP3 inflammasome assembly and IL-1β/IL-18 secretion. Expression profiling documents coordinated suppression of *hnf4a* a master regulator of hepatocyte differentiation consistent with loss of hepatocellular identity and the transition toward a dedifferentiated pro inflammatory phenotype [100].

5. Nanoparticle Induced Mitochondrial Dysfunction

5.1 Types of Nanoparticles

Metal Nanoparticles Metal nanoparticles (MNPs) including silver (AgNPs), zinc oxide (ZnO-NPs), titanium dioxide (TiO_2-NPs), copper oxide (CuO-NPs) and gold (AuNPs) constitute the most extensively studied category of the engineered nanomaterials in aquatic toxicology. Their toxicity is governed by an interplay of particle specific properties size (<100 nm), crystalline phase, surface functionalization and dissolution rate with the biological milieu. AgNPs and CuO-NPs exhibit particularly high mitochondrial toxicity mediated through metal ion release (Ag^+, Cu^{2+}) direct ETC inhibition and thiol group oxidation in mitochondrial enzymes [101]. ZnO-NPs exert toxicity via Zn^{2+} dissolution dependent disruption of mitochondrial calcium handling while TiO_2-NPs generate hydroxyl radicals upon UV activation inducing localized mtDNA strand breaks.

Carbon Nanoparticles Carbon-based nanomaterials including fullerenes (C_{60}), carbon nanotubes (SWCNTs, MWCNTs), carbon quantum dots and graphene oxide (GO) interact with biological membranes through hydrophobic intercalation and electrostatic interactions. Fullerenes and SWCNTs adsorb onto mitochondrial membranes, impeding protein import machinery and respiratory chain super complex assembly. GO elicits sharp edge physical puncture of mitochondrial membranes and activates the integrated stress response through mitochondrial unfolded protein response (UPRmt) pathways evidenced by upregulation of *atf5*, *hspd1* and *lonp1* in exposed zebrafish [102].

Polymeric Nanoparticles Polymeric NPs comprising polystyrene (PS-NPs), poly(lactic-co-glycolic acid) (PLGA) and polyamide are ubiquitous in aquatic systems as MPs degradation products and pharmaceutical delivery vehicles. PS-NPs in the 20-100 nm range exhibit efficient mitochondrial targeting due to their negative surface charge and the affinity for the positively charged mitochondrial matrix. Unlike ionic metal NPs, polymeric NPs exert toxicity predominantly through physical membrane disruption, surface mediated ROS generation and interference with the mitochondrial protein quality control systems [103].

5.2 Cellular Uptake and Bioaccumulation

Nanoparticle internalization in zebrafish tissues proceeds via size and the surface chemistry dependent pathways including clathrin mediated endocytosis, macropinocytosis and for the smallest fractions (<20 nm) passive membrane permeation. Following endosomal escape NPs traffic toward mitochondria driven by the strongly negative mitochondrial membrane potential ($\Delta\Psi m \approx -180$ mV) which electrostatically attracts cationic or the dipolar nanostructures. Inductively coupled plasma mass spectrometry (ICP-MS) quantification of zebrafish tissue digests confirms hepatic and cardiac bioaccumulation factors exceeding 1000-fold relative to ambient water concentrations for AgNPs and ZnO-NPs at 96-hour exposures. Trophic transfer studies using zebrafish feeding paradigms demonstrate biomagnification across biological membranes with nanoscale fractions penetrating

mitochondrial double membranes more efficiently than bulk sized counterparts as confirmed by energy dispersive X-ray spectroscopy (EDX) and cryo-electron tomography [104].

5.3 Mitochondrial Gene Alterations

ROS Overproduction Nanoparticle mitochondria interactions uniformly converge on excessive superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) generation primarily at Complex I and III of the ETC. Whole transcriptome analyses of NP exposed zebrafish consistently identify differential expression of mitochondrial ROS regulators including *sod2*, *prdx3*, *txnrd2* and *gpx4* with initial compensatory upregulation followed by expression collapse under chronic exposure. Correspondingly, suppression of *uqcrb* (ubiquinol-cytochrome c reductase binding protein) and *ndufv1* (NADH: ubiquinone oxidoreductase core subunit V1) mechanistically accounts for electron leak and superoxide overproduction at the semiquinone radical intermediate. Importantly, NP-induced ROS generation exhibits a threshold nonlinearity with sub-threshold exposures activating hermetic Mito hormesis pathways (*pgc-1α*, *sirt1*, *nrf2*) and suprathreshold exposures triggering irreversible bioenergetic collapse [105].

ATP Depletion Nanoparticle induced mitochondrial dysfunction results in profound ATP depletion through the convergent mechanisms like ETC uncoupling reduces the proton motive force driving ATP synthase, ROS mediated oxidative inactivation of ATPase catalytic subunits reduces enzymatic efficiency and mtDNA damage suppresses transcription of ATP synthase encoding genes *mt-atp6* and *mt-atp8*. Bioluminescent ATP assays in isolated zebrafish cardiac mitochondria confirm >60% ATP reduction following 24 hour ZnO-NP treatment at 1 mg/L with parallel transcriptional suppression of the adenylate kinase (*ak1*) and creatine kinase (*ckmt2*) key phosphotransferase enzymes maintaining cellular energy buffers. Cellular energy crisis activates AMPK which illogically inhibits mTORC1 dependent mitochondrial biogenesis establishing a feedforward cycle perpetuating energetic insufficiency [106].

Apoptosis Signalling Nanoparticle triggered mitochondrial dysfunction initiates the intrinsic apoptotic cascade through multiple converging signals: mPTP opening, cytochrome c release, Bcl-2 family protein dysregulation and caspase-9/caspase-3 activation. RNA seq data from NP exposed zebrafish heart and liver tissues document upregulation of pro-apoptotic *bax*, *bak1*, *puma* and *noxa* alongside suppression of anti-apoptotic *bcl2*, *bcl2l1* and *mcl1a*. The mitochondrial apoptosis inducing factor (*aifm1*) and the endonuclease G (*endog*) mediators of caspase independent DNA fragmentation are concurrently released from the mitochondrial intermembrane space contributing to large scale genomic degradation. TUNEL staining of zebrafish hepatic sections confirms spatiotemporally resolved apoptotic cell death correlating with mitochondrial gene dysregulation establishing a mechanistic scaffold linking NP exposure to programmed cell death in the cardiohepatic tissues [107].

5.4 Cardiohepatic Effects in Zebrafish

Cardiac Edema Pericardial and yolk sac edema are among the most consistently observed morphological phenotypes in the NP exposed zebrafish larvae arising from a confluence of mitochondria mediated cardiomyocyte dysfunction, vascular endothelial permeability disruption and the ionic imbalance. AgNPs and CuO-NP exposures at nanomolar concentrations produce dose dependent pericardial edema within 48-96 hours post-fertilization quantifiable by standardized morphometric analysis. At the molecular level the mitochondrial dysfunction in the cardiac endothelial cells suppresses claudin-5 (*cldn5b*) and occludin (*ocln*) tight junction protein expression by increasing paracellular permeability and transvascular fluid flux. Concurrent suppression of vascular endothelial growth factor receptor signalling (*kdr*, *kdrl*) impairs angiogenic repair mechanisms perpetuating oedematous phenotypes [108].

Liver Necrosis and Inflammation NP induced hepatotoxicity in the zebrafish progresses from reversible mitochondrial dysfunction to irreversible hepatocellular necrosis through a defined molecular sequence. Early phase responses include oxidative stress gene induction, mitochondrial fragmentation and ER stress activation (*atf6*, *ire1α*, *perk*) late phase responses involve NLRP3 inflammasome mediated IL-1β processing, NF-κB-driven inflammatory cytokine expression (*tnfa*, *il6*, *il1b*) and complement cascade activation. Histological grading of ZnO-NP exposed zebrafish livers reveals zonal necrosis preferentially affecting pericentral hepatocytes regions of highest CYP450 activity and the lowest oxygen tension consistent with metabolic activation of redox active Zn^{2+} as a cofactor in the radical chain reactions [109]. Transcriptional suppression of hepatocyte nuclear factor 4α (*hnf4a*) and liver receptor homolog-1 (*lrh1*) marks the transition from adaptive to maladaptive hepatic responses presaging irreversible parenchymal loss.

6. Xenobiotics and Mitochondrial Gene Responses

6.1 Definition and Classification of Xenobiotics

Xenobiotics encompass chemically diverse foreign substances not endogenously synthesized by an organism including pharmaceuticals, industrial chemicals, agricultural pesticides, food additives and the combustion byproducts. For regulatory and mechanistic purposes xenobiotics are classified by the chemical structure (organochlorines, organophosphates, polycyclic aromatic hydrocarbons [PAHs], phthalates, bisphenols and dioxins), mode of action (receptor agonists/antagonists, enzyme inhibitors, Geno toxicants and immunotoxins) and environmental persistence (recalcitrant POPs versus rapidly degrading compounds). Within the context of mitochondrial toxicogenomics xenobiotics are further stratified by primary mechanism: direct ETC inhibitors (rotenone, antimycin A), mitochondrial uncouplers (dinitrophenol, carbonyl cyanide m-chlorophenyl hydrazone [CCCP]), mtDNA-intercalating agents (ethidium bromide, PAH-DNA adducts) and indirect Mito toxicants acting through epigenetic or transcriptional mechanisms [110].

6.2 Common Xenobiotic Pollutants

Pesticides Organophosphate (OP) and organochlorine (OC) pesticides represent the most extensively documented mitochondrial toxicants in aquatic ecosystems. Chlorpyrifos, malathion and diazinon inhibit mitochondrial Complex I activity through direct binding to the ND subunit interface which uncoupling OXPHOS and elevating ROS production in zebrafish hepatic and cardiac tissues. Chronic low dose chlorpyrifos exposure in the zebrafish larvae induces transcriptional reprogramming of the mitochondrial biogenesis network suppressing *pgc-1α*, *nrf1* and *tfam* expression a triad governing coordinated nuclear and mitochondrial genome expression. OC pesticides including endosulfan and lindane insert into the inner mitochondrial membrane dissipating ΔΨm and activating Ca^{2+}-dependent mPTP producing a toxicological signature that is mechanistically indistinguishable from classical mitochondrial disease states [111].

Pharmaceuticals Pharmaceutical compounds including antibiotics (tetracyclines, fluoroquinolones), cardiovascular agents (statins, amiodarone) and anti-inflammatory drugs (diclofenac, triclosan) accumulate in aquatic environments through incomplete wastewater treatment. Tetracyclines directly inhibit mitochondrial ribosome function by binding 16S rRNA of the mito ribosome small subunit suppressing translation of all 13 mitochondrially encoded OXPHOS subunits. Amiodarone, a widely prescribed antiarrhythmic which inhibits mitochondrial Complex I and impairs fatty acid β-oxidation in the zebrafish hepatocytes through inhibition of *cpt1a* a mechanism directly underlying its clinical hepatotoxicity [112]. Statins inhibit the mevalonate pathway depleting coenzyme Q10 (ubiquinone) an obligate electron carrier in the ETC and thereby impairing mitochondrial electron transfer and promoting myopathic energy deficiency recapitulated faithfully in zebrafish cardiac tissue.

Industrial Chemicals Polychlorinated biphenyls (PCBs), bisphenol A (BPA), perfluoroalkyl substances (PFAS) and heavy metals (cadmium, mercury and arsenic) represent persistent industrial contaminants with well characterized mitochondrial modes of action. BPA activates estrogen receptor β (ERβ) and G protein coupled estrogen receptor (GPER) signalling within mitochondria altering mitochondrial gene expression through estrogen response elements identified in the D-loop region. PFAS compounds particularly PFOS and PFOA disrupt mitochondrial β-oxidation through competitive inhibition of the acyl-CoA dehydrogenase family producing a hepatic metabolic phenotype in zebrafish consistent with PFAS-associated liver disease in occupationally exposed humans. Cadmium and inorganic mercury directly inhibit thiol-dependent mitochondrial enzymes including pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, collapsing the tricarboxylic acid (TCA) cycle and electron donor supply to the ETC [113].

6.4 Altered Mitochondrial Gene Expression

Stress-Response Genes Xenobiotic exposure activates a highly conserved mitochondrial stress transcriptional program in zebrafish cardiohepatic tissues. The mitochondrial unfolded protein response (UPRmt) mediated through ATFS-1/ATF5 nuclear translocation upon mitochondrial import failure upregulates mitochondrial chaperones (*hspd1*, *hspe1*, *grpel1*) and proteases (*clpp*, *lonp1*, *yme1l1*) seeking to restore proteostasis within the organelle matrix. Concurrently, the integrated stress response (ISR) is triggered through HRI and GCN2 kinase-mediated eIF2α phosphorylation, selectively translating stress response transcription factors including ATF4 and CHOP/DDIT3. Sustained ISR activation, however, paradoxically suppresses global protein synthesis and induces apoptotic gene programs delineating the transition from adaptive to maladaptive mitochondrial stress responses in xenobiotic toxicity paradigms [114]

Apoptotic Genes Xenobiotic perturbed mitochondria release a repertoire of intermembrane space proteins that orchestrate programmed cell death. Cytochrome c release activates Apaf-1 apoptosome assembly, driving procaspase-9 autoactivation and downstream effector caspase-3/-7 proteolysis of >600 cellular substrates a cascade transcriptomically reflected in zebrafish by coordinated upregulation of *casp3a*, *casp7*, *casp9* and *apaf1*. Second mitochondria-derived activator of caspases (SMAC/Diablo) antagonizes inhibitor of apoptosis proteins (IAPs), amplifying caspase activation, while AIF and EndoG translocate to nuclei to execute caspase-independent large scale chromatin fragmentation. Whole mount TUNEL analysis in xenobiotic exposed zebrafish larvae spatially resolves apoptotic hotspots to hepatocyte and cardiomyocyte populations confirming mitochondria dependent cell death as the primary effector mechanism of cardiohepatic xenobiotic toxicity [115].

Energy Metabolism Genes Global transcriptomic analyses of xenobiotic exposed zebrafish consistently identify suppression of nuclear encoded genes governing mitochondrial energy metabolism, including *sdha*, *uqcrc2*, *cox5a*, *atp5f1a* and the entire NDUF gene family encoding Complex I subunits. Parallel suppression of *pgc-1α* the master transcriptional coactivator of mitochondrial biogenesis reduces mitochondrial mass, OXPHOS capacity and fatty acid oxidation flux compounding the direct ETC inhibitory effects of xenobiotics. Glycolytic compensation is transiently upregulated through HIF-1α stabilization and transcriptional induction of *hk2*, *pfkla* and *ldha* but proves insufficient to sustain ATP homeostasis in energetically demanding cardiac tissue [116]. This bioenergetic reprogramming from OXPHOS dependent aerobic metabolism to glycolysis dependent anaerobic glycolysis recapitulates the Warburg like metabolic shift increasingly recognized as a convergent response to mitochondrial dysfunction across diverse toxicological and pathological contexts.

8. Multi-Omics Approaches in Mitochondrial Toxicology

8.1 Transcriptomics

Whole transcriptome RNA sequencing (RNA-seq) of toxicant exposed zebrafish hearts and livers provides unbiased genome wide resolution of mitochondrial gene regulatory networks at single nucleotide sensitivity. Differential expression analysis routinely identifies hundreds to thousands of mitochondrially relevant transcripts spanning nuclear encoded ETC subunits, mitochondrial dynamics regulators and stress-response effectors,

enabling construction of toxicant specific molecular signatures. Single-cell RNA seq (scRNA-seq) now resolves transcriptional heterogeneity within cardiac and hepatic cell populations distinguishing cardiomyocyte, endothelial, stellate cell and hepatocyte specific mitochondrial responses overcoming the limitation of bulk analyses that mask cell type specific vulnerabilities. Spatially resolved transcriptomics (10x Visium, Slide-seq) further localizes mitochondrial gene expression changes to discrete anatomical zones (hepatic pericentral vs. periportal regions; ventricular vs. atrial myocardium) with histological precision providing mechanistic context for the zonal toxicity patterns observed in zebrafish histopathology [117].

8.2 Proteomics

Mitochondria enriched proteomics using isobaric tandem mass tag (TMT) labelling and data independent acquisition (DIA) mass spectrometry enables quantification of >2000 mitochondrial proteins from zebrafish cardiohepatic tissues, resolving post translational modifications (phosphorylation, acetylation, succinylation, glutathionylation) that govern OXPHOS complex assembly and activity beyond transcriptional regulation. Phosphoproteomic analyses of toxicant-exposed zebrafish have identified PKA and AMPK mediated mitochondrial protein phosphorylation networks that dynamically modulate respiratory chain super complex assembly under energetic stress. Interactomics approaches co-immunoprecipitation coupled with proximity-labelling proteomics (BioID, TurboID) map toxicant induced disruption of protein–protein interaction networks within ETC super complexes revealing novel complex I–III–IV interaction interfaces as targets of environmental contaminants. Integration of mitochondrial proteomics with transcriptomics datasets through systems biology workflows resolves post transcriptional regulatory nodes ubiquitin proteasome degradation, mito ribosome assembly factors that are invisible to transcriptional analysis alone [118].

8.3 Metabolomics

Targeted and untargeted metabolomics by liquid chromatography mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy profiles the functional output of mitochondrial metabolic networks in toxicant exposed zebrafish with comprehensive coverage. Isotope tracing using ^{13}C-labeled glucose, glutamine and fatty acids combined with GC-MS enables flux quantification through the TCA cycle, ETC and β-oxidation pathway distinguishing toxicant induced suppression of flux from compensatory rerouting through alternative pathways. Toxicant specific metabolic fingerprints have been established in zebrafish ETC inhibitors produce succinate and NADH accumulation with citrate depletion, β-oxidation inhibitors yield acylcarnitine accumulation detectable by tandem MS and mtDNA damaging agents produce global OXPHOS metabolite depression alongside elevated lactate/pyruvate ratios reflecting cytoplasmic redox compensation [119]. Mitochondrial metabolomics data integrated with transcriptomics and proteomics through constraint-based metabolic network modelling (flux balance analysis) enables mechanistic attribution of metabolic phenotypes to specific enzyme level perturbations advancing mechanistic toxicology beyond correlative biomarker identification.

8.4 Epigenetic Regulation of Mitochondrial Genes

Epigenetic mechanisms DNA methylation, histone modification and non-coding RNA regulation increasingly emerge as critical mediators of environmentally induced mitochondrial gene dysregulation with transgenerational implications for cardiohepatic health. Whole genome bisulfite sequencing (WGBS) of toxicant exposed zebrafish identifies differentially methylated regions (DMRs) in the promoters of *pgc-1α*, *tfam*, *nrf1* and nuclear encoded ETC subunit genes with hypermethylation correlating with the transcriptional silencing persisting beyond acute exposure windows. Histone H3K27 trimethylation (H3K27me3) at mitochondrial biogenesis gene loci deposited by Polycomb Repressive Complex 2 (PRC2) activated by toxicant induced ROS establishes heritable chromatin states that suppress mitochondrial gene expression across subsequent cell divisions. Within the mitochondrial matrix itself direct cytosine methylation of mtDNA at non CpG sites by DNMT3B has been documented in toxicant exposed zebrafish with functional consequences for mitochondrial transcription factor binding and the gene expression. Toxicant induced changes in mitochondria derived metabolites (acetyl-CoA, α-ketoglutarate, SAM, NAD^+) substrates for chromatin modifying enzymes establish a bidirectional coupling between the mitochondrial metabolic state and nuclear epigenetic landscape that propagates mitochondrial dysfunction into stable transcriptional alterations transmissible to progeny [120].

9. Biomarkers and Therapeutic Perspectives

9.1 Mitochondrial Biomarkers of Toxicity

A healthy panel of mitochondrial biomarkers has been validated in the zebrafish for environmental toxicant assessment, spanning genomic, biochemical and morphological dimensions. At the genomic level mtDNA copy number (quantified by droplet digital PCR) or 8-OHdG content and the 4977 bp common deletion frequency provide quantitative indices of mtDNA integrity and the replicative stress. Biochemical biomarkers including ETC complex activities (Complexes I-V spectrophotometric assays), citrate synthase activity (mitochondrial mass surrogate), ΔΨm (JC-1, TMRE fluorescence) and cellular ATP content (bioluminescence assay) offer functional resolution of bioenergetic status [121]. Circulating biomarkers plasma cell free mtDNA, mitochondrial DAMPs (mtDNA, TFAM, cardiolipin) and metabolite ratios (lactate/pyruvate, acylcarnitine profiles) enable minimally invasive systemic monitoring of mitochondrial stress in zebrafish through collection of pooled media or hemolymph equivalents. Transcriptomic biomarker panels comprising *pgc-1α*, *tfam*, *mt-nd1*, *mt-co1*, *sod2* and *casp3a* expression ratios have been validated across multiple toxicant classes, offering transcriptome based hazard screening capacity applicable to high throughput chemical testing programs.

9.2 Early Detection Strategies

Zebrafish offer unparalleled capacity for high-throughput early detection through the integration of automated phenotypic screening with molecular endpoint quantification. Automated imaging platforms (Image Xpress, Operetta CLS) coupled with machine learning-based image analysis enable rapid, unbiased quantification of cardiac morphology, pericardial area, liver pigmentation and tail curvature as early indicators of mitochondrial dysfunction across thousands of larvae per day. Genetically encoded mitochondrial biosensors including mito-roGFP2 (redox status), Mito Timer (protein turnover) and mito-APEX2 (proximity proteomics) expressed in transgenic zebrafish lines enable real time, non-invasive monitoring of mitochondrial redox state, biogenesis dynamics and protein microenvironment with subcellular resolution. Multi parametric flow cytometry of dissociated zebrafish tissues simultaneously quantifies ΔΨm, ROS levels, mitochondrial mass, apoptosis (Annexin V) and cell viability in individual cells providing high-content mitochondrial profiling from nanogram scale tissue samples [122]. Integration of these detection modalities within tiered testing frameworks combining rapid morphological screening with targeted molecular validation provides cost effective early identification of mitotoxic environmental contaminants.

9.3 Antioxidants and Protective Compounds

Pharmacological intervention studies in zebrafish have demonstrated protective efficacy for multiple antioxidant and mitochondria-targeted compounds against cardiohepatic toxicant injury. MitoQ a ubiquinone-triphenyl phosphonium conjugate that accumulates ~1000-fold within the mitochondrial matrix driven by ΔΨm attenuates NP induced ETC dysfunction, apoptosis and cardiac edema in zebrafish at nanomolar concentrations establishing mitochondria targeted antioxidants as mechanistically superior to untargeted approaches. N-acetylcysteine (NAC) replenishes glutathione pools depleted by toxicant exposure, restoring redox homeostasis and reducing mtDNA oxidative damage with demonstrated efficacy in zebrafish pesticide and heavy metal toxicity models [123]. Resveratrol and quercetin activate SIRT1/PGC-1α signalling, stimulating mitochondrial biogenesis to compensate for toxicant induced mitochondrial mass depletion and have demonstrated partial rescue of hepatic lipid accumulation and cardiac function in zebrafish MP and xenobiotic exposure models. Coenzyme Q10 supplementation restores ETC electron carrier pools depleted by statin and heavy metal exposures, while SS-31 (elamipretide) a mitochondria targeting peptide that binds cardiolipin preserves cristae architecture and respiratory super complex integrity under oxidative stress representing a mechanistically targeted protective strategy with translational therapeutic potential [124].

10. Human Health and Environmental Implications

Translational Relevance to Human Diseases The zebrafish cardiohepatic mitochondrial toxicity paradigm established through MP, NP and xenobiotic research carries direct translational relevance to human diseases of rising global burden. Mechanistic parallels between environment induced mitochondrial dysfunction in zebrafish and the pathophysiology of human non-alcoholic fatty liver disease (NAFLD), dilated cardiomyopathy and metabolic syndrome are supported by conservation of key molecular pathways Nrf2/NF-κB, PINK1/Parkin, PGC-1α/SIRT1, Bcl-2/Bax across vertebrate species. Epidemiological studies in human populations document associations between elevated blood/urine MP levels, heavy metal burden and biomarkers of mitochondrial dysfunction (circulating cell-free mtDNA, ΔΨm, mtDNA heteroplasmy) suggesting that the mechanistic insights derived from zebrafish models are recapitulated in environmentally exposed human cohorts [125]. The zebrafish model's pharmacological tractability further enables identification of therapeutic agents mitochondria targeted antioxidants, PINK1 activators, NAD^+ precursors that can be rapidly advanced to mammalian efficacy and safety testing accelerating the drug development pipeline for environment linked mitochondrial disease.

Ecotoxicological Significance The concentrations of MPs, NPs and xenobiotics at which mitochondrial toxicity is documented in zebrafish increasingly overlap with environmental measurements in surface waters, sediments and biological tissues of wild fish populations attributing immediate ecotoxicological significance to these findings. Sublethal mitochondrial impairment manifesting as altered swimming behaviour, the reproductive suppression and immunocompromise at the environmentally relevant concentrations may contribute to population level fitness declines observed in wild aquatic vertebrates inhabiting contaminated ecosystems [126]. Trophic transfer studies demonstrate bioaccumulation and biomagnification of mitotoxic contaminants through the aquatic food webs with zebrafish derived bioaccumulation factors informing ecologically protective water quality guidelines and risk assessment frameworks. The integrative use of zebrafish mitochondrial biomarkers in the environmental monitoring programs complementing physicochemical water quality parameters offers a biologically relevant early warning system for ecosystem health degradation driven by the emerging contaminants [127].

Risk Assessment Perspectives Traditional risk assessment frameworks based on the acute lethality endpoints (LC_{50}) systematically underestimate the toxicological significance of sublethal mitochondrial dysfunction occurring at orders of magnitude lower concentrations [128]. Integration of zebrafish mitochondrial endpoints ETC activity, ΔΨm and mtDNA copy number transcriptomic pathway perturbation scores into computational toxicology platforms (Tox21, ToxCast) enables derivation of the mechanistically anchored benchmark concentrations for environmental risk characterization. Adverse outcome pathway (AOP) frameworks connecting molecular initiating events (ETC inhibition, mtDNA oxidation, ΔΨm collapse) through intermediate key events (ATP depletion, ROS overproduction, apoptosis) to adverse outcomes (cardiac edema, hepatic steatosis, population decline) which provide regulatory compatible mechanistic scaffolds for the risk extrapolation from zebrafish to ecological and human receptors. Mixture toxicity assessment incorporating concentration addition

and independent action models to address the co-occurrence of MPs, NPs and xenobiotics in the real environmental matrices represents an essential frontier for realistic exposure characterization and cumulative risk quantification [129].

11. Challenges and Future Directions

Knowledge Gaps Despite significant advances critical knowledge gaps make mechanistic understanding of the environmental mito toxicity in the zebrafish. The mitochondrial proteome remains incompletely characterized in zebrafish cardiac and hepatic tissues across developmental stages limiting interpretation of proteomic perturbation data in the context of organelle level functional organization. Interactions between mitochondrial toxicants and the mitochondria associated endoplasmic reticulum membrane (MAM) a specialized interface governing Ca^{2+} transfer, lipid metabolism and apoptotic signalling remain minimally explored in the zebrafish toxicology representing a significant mechanistic blind spot. Sex specific differences in zebrafish mitochondrial toxicant sensitivity which documented for several heavy metals and EDCs require systematic investigation given the established dimorphism of mitochondrial biogenesis and the bioenergetic capacity between male and female vertebrates.

Need for Long Term Exposure Studies The preponderance of existing zebrafish mitochondrial toxicity data derives from acute (24-96 hrs) larval exposures that do not recapitulate the chronic multigenerational nature of real world environmental contamination. Long term exposure paradigms spanning zebrafish adulthood (3-24 months) are essential to characterize mitochondrial adaptive remodelling, epigenetic drift, clonal expansion of mtDNA mutants and the progressive cardiohepatic fibrosis that develop only over extended exposure windows. Multigenerational exposure studies exposing F0 animals and tracking F1-F3 progeny are critically needed to assess whether toxicant induced mitochondrial epigenetic modifications (*pgc-1α* hypermethylation, H3K27me3 at ETC gene loci) are trans generationally heritable a question with profound implications for population level ecotoxicology and human ancestral exposure risk.

Advanced Imaging and Omics Technologies Emerging technologies offer transformative capacity to resolve outstanding questions in zebrafish mitochondrial toxicology. Cyro electron tomography of toxicant-exposed zebrafish cardiac mitochondria will achieve near-atomic visualization of respiratory super complex disassembly, cristae remodelling and mPTP structural determinants under pathophysiological conditions. Spatial multi-omics platforms integrating transcriptomics, proteomics and metabolomics with single cell spatial resolution such as MERFISH, seq FISH+ and spatial proteomics by MIBI-TOF will map the cellular topology of mitochondrial dysfunction within intact zebrafish tissue sections. Single mitochondrion omics isolating individual organelles by microfluidics for genome, transcriptome or proteome analysis will resolve the intra cellular mitochondrial population heterogeneity that is entirely obscured by conventional bulk analyses, enabling characterization of the clonal dynamics of mtDNA mutation accumulation in toxicant exposed zebrafish tissues.

12. Conclusion

Summary of Major Findings This review has systematically established that microplastics, engineered nanoparticles, and environmental xenobiotics converge on mitochondria as a primary cellular target in zebrafish cardiohepatic toxicity, perturbing a core set of mitochondrial gene networks governing OXPHOS integrity, redox homeostasis, biogenesis, dynamics and apoptotic commitment. Across diverse toxicant classes shared molecular signatures include suppression of the PGC-1α/NRF1/TFAM biogenesis triad, transcriptional repression of nuclear and mitochondrially encoded ETC subunit genes, dysregulation of mitochondrial fusion-fission dynamics and activation of the intrinsic apoptotic cascade through cytochrome c release and caspase-mediated proteolysis. Toxicant-specific mechanistic fingerprints metal ion-mediated enzyme inhibition for MNPs, physical membrane intercalation for MPs and polymeric NPs and CYP-mediated bioactivation for xenobiotics overlay these shared pathways generating toxicant class-specific nuances that inform mechanism-based hazard characterization. Multi-omics integration transcriptomics, proteomics, metabolomics, and epigenomics applied in zebrafish has revealed the full regulatory architecture of toxicant-induced mitochondrial dysfunction extending from primary molecular initiating events through downstream systems level metabolic and transcriptional reprogramming to organismal cardiohepatic pathology.

Future Scope of Zebrafish Mitochondrial Toxicogenomics Research Zebrafish mitochondrial toxicogenomics stands at an inflection point poised to transition from descriptive mechanistic characterization to mechanistically-grounded regulatory science, therapeutic target discovery and precision environmental health applications. The integration of CRISPR engineered mitochondrial disease models, spatial multi omics, AI driven data integration and the organ on chip platforms within the zebrafish toxicogenomics programs will generate mechanistic depth and predictive resolution unprecedented in the history of environmental toxicology. Establishing internationally harmonized zebrafish mitochondrial toxicogenomics testing guidelines incorporating validated biomarker panels, the standardized exposure protocols and multi-omics data reporting standards will catalyse regulatory adoption of these mechanistically superior approaches in the chemical risk assessment frameworks globally. Ultimately, the zebrafish mitochondrial toxicogenomics paradigm offers a scientifically difficult, ethically advantageous and the translationally powerful platform for advancing understanding of how the contaminated environment erodes mitochondrial genome integrity and through it cardiovascular and hepatic health across species from aquatic ecosystems to human populations.

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