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MINI REVIEW

ZEBRAFISH MALFORMATIONS AND NEUROBEHAVIORAL EFFECTS INDUCED BY HEAVY METALS EXPOSURE AT DIFFERENT DEVELOPMENTAL STAGES - A MINI-REVIEW

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Abstract. *Heavy metal exposure poses significant risks to aquatic organisms, including zebrafish, leading to various malformations and neurobehavioral effects across different developmental stages. This mini-review explores the impact of heavy metals, such as arsenic (As), cadmiu (Cd), lead (Pb), zinc (Zn), iron (Fe), copper (Cu) and others on zebrafish, focusing on the toxicological effects during embryonic, larval, and adult stages. Exposure to heavy metals during various developmental stages of zebrafish leads to significant malformations and neurobehavioral effects. The review discusses how these metals induce neurobehavioral effects by disrupting key biochemical processes, altering neurotransmitter systems, and increasing oxidative stress, ultimately leading to impairments in different behavior patterns.*

Keywords: heavy metals, zebrafish, neurobehavioral effects, developmental stages

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Introduction

Heavy metals, defined as substances with a density at least five times that of water, can be either essential or non-essential based on their role in cellular functions. Despite their density and essentiality, even small amounts can cause toxicity. Non-essential metals are especially toxic at low concentrations, affecting metabolic pathways. They bioaccumulate, leading to greater damage with prolonged exposure. Pollution from mining, industry, and agriculture are major sources, with natural forces like volcanic eruptions also contributing [1]. These metals can accumulate in the environment, causing long-term damage and posing serious health risks such as neurological disorders, respiratory issues, and other chronic diseases [2].

Research on the adverse effects of toxicants has focused on studying individual components, even though organisms are usually exposed to combinations of toxic agents. There is limited understanding of the developmental effects of exposure to mixtures of heavy metals. An added concern with mixture exposure is the potential amplification of toxicity, where the combined effects of multiple toxicants could be greater than the sum of their individual effects. This amplification is particularly expected for compounds with similar modes of action, which could interact in an additive or synergistic manner [3]. There is a growing link between exposure to heavy metals and neurodegeneration, which poses a public health concern due to the rising prevalence of dementia, the negative impacts of neurodegeneration-related disabilities, and increasing environmental pollution. Exposure to highly toxic metals such as cadmium, mercury, and lead can cause neurological deficiencies in the human body, including memory loss, paralysis, mental disorders, and paraesthesia. Neurotoxicity often leads to neurodegenerative diseases [4].

Early developmental stages of fish, particularly embryos and larvae, are more vulnerable to pollutants like heavy metals compared to juvenile and adult fish. As a result, they are commonly used as bio-indicators to assess the toxicity of these chemicals in aquatic organisms. Various endpoints, such as developmental malformations (teratogenicity), physiological and biochemical changes, and behavioral and functional deformities, are utilized to evaluate the toxicity of heavy metals to fish populations. Fish embryos and larvae at different developmental stages (blastula, gastrula, segmentation, hatching, etc.) respond differently to exposure, depending on factors such as species, type of metal, mode of action, concentration of metals, and exposure duration [5].

The Relevance of Heavy Metal Exposure

Pollution sources include mining waste, landfill leachates, municipal and industrial wastewater, urban runoff, and natural events like volcanic eruptions, weathering, and rock abrasion. Heavy metal ions are toxic, potentially

carcinogenic, and have the ability to bioaccumulate in living organisms [6]. When these metals are indiscriminately released into water bodies, they cause significant ecological damage and pose lethal risks to non-target organisms. The contamination disrupts the food chain, threatening biodiversity and the overall ecological balance. In waters with high levels of pollution, fish and other aquatic organisms are particularly vulnerable, as indicated by the presence of heavy metal residues in fish [7, 8]. At low concentrations, non-essential metals can be toxic even in small amounts. In the environment, heavy metals do not break down, making them some of the most persistent and non-biodegradable pollutants. These metals create a cyclical contamination chain within ecosystems, affecting the atmosphere, soil, water, fish, and ultimately humans [9].

Heavy metals are classified as trace elements due to their presence in trace amounts (from the ppb range to less than 10 ppm) in various environmental matrices. Their bioavailability is influenced by physical factors such as temperature, phase association, adsorption, and sequestration. Chemical factors, including speciation at thermodynamic equilibrium, complexation kinetics, lipid solubility, and octanol/water partition coefficients, also impact their availability. Additionally, biological factors, such as species characteristics, trophic interactions, and biochemical/physiological adaptations, significantly contribute to their behavior and effects [10]. The term “heavy metals” lacks a universally agreed-upon definition, but it generally refers to metals and metalloids with relatively high densities (greater than 5 g/cm3), the ability to bioaccumulate through the food chain, and typically high toxicity to living organisms. Some experts recommend replacing the term “heavy metals” with “potentially toxic elements” to avoid confusion. This category includes harmful metals such as cadmium (Cd), lead (Pb), nickel (Ni), chromium (Cr), mercury (Hg), and metalloids like arsenic (As), which can originate from both natural sources and industrial activities [11].

Metals such as cobalt (Co), copper (Cu), Cr, iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), Ni, selenium (Se), and zinc (Zn) are essential nutrients required for various biochemical and physiological functions. A deficiency in these micronutrients can lead to a range of deficiency diseases or syndromes. However, metals like Cu can be toxic in excess, leading to diseases like Wilson’s disease. Non-essential metals, including aluminum (Al), antimony (Sb), As, barium (Ba), beryllium (Be), bismuth (Bi), Cd, gallium (Ga), germanium (Ge), gold (Au), indium (In), Pb, lithium (Li), mercury (Hg), platinum (Pt), silver (Ag), strontium (Sr), tellurium (Te), thallium (Tl), tin (Sn), titanium (Ti), vanadium (V), and uranium (U), have no known biological functions and are considered toxic [10]. The permissible levels of various heavy metal ions, established by the World Health Organization (WHO) and the European

Medicines Agency (EMA), range from ppt to ppm. As of June 1, 2020, As, Cd, Pb, Hg are among the 10 chemicals identified as major public health concerns, as highlighted on the WHO website. Despite the well-documented toxicity of these elements, their continued use in technological, medical, and agricultural applications still poses a significant threat to human health [11].

Developmental Stages of Zebrafish and Teratogenic Effects of Heavy Metals Exposure

Research on the developmental toxicity of zebrafish has become increasingly important in assessing the potential harmful effects of substances, drugs, and environmental pollutants, such as heavy metals, on the early stages of development and maturation. The most common signs of developmental toxicants in both humans and animals include: (1) altered growth, (2) structural abnormalities, and (3) functional impairments [12].

Zebrafish go through three distinct developmental stages—embryonic, larvae, juvenile, and adult—which make them ideal candidates for studies in aquatic ecotoxicology. When exposed to toxic substances, various harmful effects are observed, including death, delayed hatching, and physical deformities such as abnormal egg condensation or changes in morphology. These changes serve as key indicators in toxicity assessments [13].

Zebrafish embryos are an excellent model for studying early development due to their rapid growth and clear visibility. This makes it possible to observe key developmental processes such as motility, gastrulation, segmentation, blastomere cleavage, organ formation, and the development of the central nervous system. Additionally, the expression of receptors and the formation of the circulatory system can be easily tracked [12]. The well-defined stages of development in zebrafish enable precise assessments of how substances impact these critical developmental phases. Due to their rapid development and clear visibility, zebrafish embryos allows for the easy detection of developmental abnormalities, being highly valuable for teratogenicity testing [14]. Zebrafish embryos are particularly vulnerable to toxins between 24-72 hours post-fertilization (hpf). This stage is critical for organ development, making it a key period for evaluating toxicity [12]. Zebrafish larvae, particularly between 72 and 120 hours post-fertilization (hpf), are highly sensitive to environmental toxins, including heavy metals. During this developmental period, critical organs such as the central nervous system, eyes, and digestive system begin to form and mature. This stage is crucial for evaluating the effects of toxins, as any exposure during this time can result in malformations and developmental delays. Common malformations observed in larvae include issues like delayed hatching [15], impaired eye development [16], abnormal spinal curvature [17] and irregularities

in organ formation [18]. The vulnerability of zebrafish larvae during this period makes them an excellent model for studying developmental toxicity, as even low concentrations of pollutants can have significant impacts on their growth and survival. In juvenile and adult stages, zebrafish can show more complex responses to heavy metal exposure. Toxicity can affect the central nervous system, impair behavior, and disrupt feeding and swimming [19]. These stages are important for studying long-term effects of pollutants and their ecological and health risks. Common endpoints in developmental toxicity studies include mortality, developmental abnormalities (such as malformations and growth retardation), and behavioral changes. Specific developmental parameters, such as mortality rate, hatching rate, spontaneous and induced movement, as well as morphometric measurements like body length, eye size, yolk sac area, trunk area, and tail area, are also evaluated [12].

In Table 1, several developmental stages of zebrafish exposed to two significant heavy metals, As and Cd, are synthesized.

Table 1. Developmental Stages of Zebrafish Exposed to As and Cd.

Heavy Metal	**Developmental Stage**	**Studied Concentration**	**Exposure Time (h/d)**	**Observed Malformations**	**Reference Study**
Arsenic (As)	Embryo	0–10.0 mM	4-120hpf	-concentrations below 0.5 mM did not significantly impact embryo survival or early development -concentrations between (0.5–10.0 mM): -reduced survival -delayed hatching, stunted growth, and morphological changes -weakened tactile response to light (2.0–5.0 mM, 30 hpf) -spinal cord malformations and disordered motor axon projections (2.0 mM, 48 hpf) -bradycardia (slow heart rate) at 0.5–2.0 mM (60 hpf) -altered ventricular shape at 2.0 mM (48 hpf)	[20]

	Embryo	0, 25, 50, 75, and 150 μg/L	120 hpf	-low concentration of arsenite decreased the survival rate of zebrafish larvae	[21]
	Embryo	1 mM	60-72 hpf	-congenital deformities especially cardiac malformations in zebrafish larvae after 24 and 48 hpf	[22]
	Embryo	100□nM to 600□nM 100□nM to 600□nM 100□nM to 600□nM 100nM - 600nM	48-72-96 hpf	-hatchability, morphological abnormalities, mortality, behavioral modifications (touch induced escape response (TIER), startle response (SR) and turning behaviour (TB)	[23]
	Larvae	30–40 μM	11 dpf	-30–40 μM significantly reduces tail-coiling movements and heart rate in early larvae -at 6 days post-fertilization (dpf): motor behavior deficits but no significant changes in anxiety-like response -at 11 dpf: slight impairment in color preference, which became more pronounced in adulthood	[24]
Cadmium (Cd)	Embryo	0.07, 0.7, 3.1, and 6.6 μM	4-72 hpf	-increased yolk sac area at high cadmium concentrations, indicating metabolic rate alterations	[25]
	Embryo	16.7 μM	96 h	-exposure caused a delay in hatching and slowed yolk sac absorptio -smaller cephalic region area -reduced eye and otic vesicle area -shortened eye-snout	[26]

				distance, notochord, and embryo length -reduced swimming bladder area -enlarged pericardial sac -lower heart rate, but increased stroke volume and cardiac output	
	Embryo	9 μM	24 h	-increase in apoptotic events was observed in the brain of embryos after just 24 hours of cadmium (Cd) exposure, indicating early cellular damage	[27]
	Embryo	1 to 1,000 μM	18 h	-head and eye hypoplasia -hypopigmentation -cardiac edema -yolk sac abnormalities -altered axial curvature -tail malformations -the frequency of malformations increased with cadmium concentration -spinal deformity in embryos with altered axial curvature was linked to reduced myotome formation	[28]
	Lavae	2, 5, 10 and 50 μg/L	5 dpf	-exposure to 10 μg/L Cd inhibited body segmentation growth and skeletal mineralization development by 29.1%–56.7% -damaged the swimming ability of zebrafish and increased their sensitivity to environmental stress	[29]

Neurotoxic Behavior Induced by Heavy Metals

Zebrafish have become a fundamental model for studying neurobehavioral processes in vertebrates at the molecular level. Today, zebrafish are widely used to investigate human-relevant neurobehavioral traits. Their responses to various toxic substances are easier to assess in controlled laboratory settings, where behavioral tests—such as aggression, anxiety, and exploration—help evaluate the effects of contaminants on traits crucial for survival and reproduction. These tests are conducted on both larvae and adult zebrafish, showing that even low concentrations of neurotoxicants can significantly affect behavior. This makes zebrafish an important model in ecotoxicological studies [30].

While certain metals like Fe, sodium (Na), potassium (K), Cu, Zn, Ca, and Mg are essential for normal biological functions, they can become toxic at elevated levels. In contrast, heavy metals such as Pb, As, Cd, Cr, and Hg are highly toxic even at low concentrations due to their persistence, bioaccumulation, and chemical stability. These metals are listed among the most hazardous substances, with the World Health Organization identifying Pb, As, Cd, and Hg as major public health concerns [31].

As and Cd are both neurotoxic metals linked to several neurological disorders. As consumption impairs cognitive function and increases the risk of neurodegenerative diseases by disrupting synaptic transmission and neurotransmitter balance. It induces apoptosis in neural cells through the MAPK signaling pathways (ERK2, JNK, p38) and triggers an increase in intracellular Ca, further promoting cell death. As also activates autophagy, mediated by AMPK and inhibited by mTOR. Similarly, Cd exposure leads to neurodegenerative diseases such as ALS, Parkinson's, and Alzheimer's. It causes peripheral neuropathy, learning disabilities, and motor function impairment by inducing apoptosis in neural cells. This effect is driven by disruption of neurogenesis, inhibition of neuron gene expression, and endocrine disruption [32]. As exposure has been extensively shown to negatively affect the nervous system in rodent models, often leading to behavioral disorders such as hyperactivity and impairments in learning and memory. Although the precise mechanisms of As-induced neurotoxicity are not fully understood, they are strongly linked to increased production of reactive oxygen species (ROS), depletion of brain antioxidant enzymes, and oxidative damage to lipids and proteins. However, because aquatic species like fish exhibit behavior patterns distinct from terrestrial animals, it is essential to study how chronic As exposure alters behavior specifically in fish models [33]. Cd exposure has been shown to disrupt multiple physiological systems in zebrafish, including metabolism, cardiovascular function, behavior, and the nervous system. It induces developmental toxicity and reduces larval activity, partly by downregulating key genes involved in neuronal

development, such as nrxn2aa and nrxn2ab. Cd chloride treatment significantly increases the production of reactive oxygen species (ROS), which compromise cell membrane integrity and negatively impact the heart and blood vessels. Additionally, Cd can alter brain structure by promoting neuronal apoptosis through the mitochondrial pathway [34].

Proper metal homeostasis is essential for maintaining vital physiological functions, especially in the brain. While many metals like Fe, Cu, and Zn are crucial for neuronal activity and survival, their imbalance or excessive accumulation can lead to severe neurotoxicity. This dysregulation is linked to the development and progression of various neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, ALS, and Wilson's disease. Research highlights how metals contribute to oxidative stress, disrupt neurotransmission, and promote neuronal death, underlining their dual role as both essential nutrients and potential neurotoxic agents [35]. It has been demonstrated that Fe supplementation helps alleviate oxidative stress, brain alterations, and behavioral changes induced by heavy metals and imidacloprid in zebrafish, with honey enriched with additives, including iron, showing potential in reducing oxidative damage and enhancing brain functio [36]. Cu, even at low and environmentally relevant concentrations (μg/L range), can significantly affect the nervous system of fish. It impairs neuromodulation by reducing the activity of brain enzymes such as nucleotidases and cholinesterase. Cu also disrupts chemosensory and mechanosensory systems by damaging olfactory neurons and lateral line mechanoreceptors, which are critical for behaviors like predator avoidance and navigation. These neurochemical disturbances lead to behavioral impairments, including reduced olfactory sensitivity and increased vulnerability to predation, highlighting Cu's potent neurotoxicity even at sublethal levels [37]. Zn exposure has been shown to be neurotoxic and can lead to neurodegeneration in the central nervous system (CNS). High levels of cytosolic Zn^{2+}, transported through Ca^{2+} channels, trigger a series of downstream effects that cause neuronal cell death. Even at low concentrations (400-600 nM), Zn^{2+} can significantly increase toxicity, impairing the activity of important glycolytic enzymes like glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and phosphofructokinase. Additionally, elevated intracellular Zn^{2+} levels can increase proton concentrations, disrupting neuron function. Zn has been implicated in the progression of neurodegenerative diseases such as Alzheimer's disease, vascular dementia, and Parkinson's disease [38].

Pb and As are known to cause anxiety-like behavior and cognitive impairments in animal models, such as mice. While some studies have examined their combined developmental toxicity in zebrafish, their joint neurotoxic effects remain insufficiently understood. The nervous system is particularly vulnerable to heavy metals, and even individual exposure to Pb or As results in significant

neurotoxicity. However, it is still unclear whether their combined exposure worsens neurological damage in zebrafish. Given their widespread presence and overlapping mechanisms of neurotoxicity, it is crucial to further investigate their combined effects and underlying pathways [39]. Numerous studies have explored the neurotoxicity of Pb, revealing that it operates through multiple mechanisms, including oxidative stress, disruption of neurotransmitter systems, and the induction of cellular autophagy and apoptosis [40]. Despite reductions in global lead levels, environmental Pb exposure remains a concern, particularly for aquatic systems. Neurotoxicity is often overlooked in risk assessments, which typically focus on growth and survival. However, studies in zebrafish have shown that lead exposure—at both developmental and adult stages—induces significant behavioral and neurological effects. These include hyperactivity, memory and learning deficits, impaired exploratory behavior, anxiety-like responses, and altered startle/escape reactions. These behavioral changes are often dose-dependent and are associated with disruptions in neurotransmitter levels, reduced acetylcholinesterase (AChE) activity, hormonal imbalances, and altered expression of genes involved in neurodevelopment, neurotoxicity, and stress [31].

Conclusions

In conclusion, exposure to heavy metals significantly impacts zebrafish, causing both neurobehavioral effects and physical malformations. These metals disrupt normal development at various stages, leading to impairments in locomotion, memory, and social behavior. Neurobehavioral alterations, such as reduced exploration, hyperactivity, and memory deficits, are often linked to oxidative stress, neurotransmitter imbalances, and cellular damage. Additionally, heavy metal exposure can cause malformations, including changes in body morphology, organ development, and neuronal structure. The cumulative effects of these toxic substances highlight the need for further research to understand the mechanisms behind these disruptions and their long-term implications for aquatic ecosystems and human health.

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