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Lead (Pb)-Induced Neurotoxicity in Zebrafish (*Danio rerio*): A Narrative Review of Mechanisms, Biomarkers and Behavioral Outcomes

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Abstract **Background:** Lead (Pb) is present in the environment and is a neurotoxic substance that has important implications for global public health and ecosystem stability. Given its high genetic similarity and short developmental time, the zebrafish (*Danio rerio*) has become a model organism of choice to study these effects. A clear mechanistic link between molecular triggers and functional neurobehavioral deficits is crucial to improve environmental risk assessment. **Methods:** Literature published in PubMed, Scopus and Web of Science (2013-2026) was used for the synthesis, which was organized into a structured narrative format. This review concentrated on the molecular signalling, developmental biomarkers and the changes in behaviour induced by exposure to Pb that have been studied during the past decade. **Results:** Oxidative stress is synthesized evidence as a major initiating event that leads to involvement of mitochondrial dysfunction, lipid peroxidation and apoptotic signaling. The main molecular alterations are related to alterations of dopaminergic and cholinergic pathways and they are related to a specific ontogenetic transition - hyperactivity in the larvae and anxiety-like behavior and cognitive impairment in the adults. Markers of acute stress, like Heme Oxygenase-1 (HO-1) and Brain-Derived Neurotrophic Factor (BDNF), are sensitive markers but new evidence points to transgenerational risks through epigenetic remodeling. **Conclusions:** Zebrafish is a powerful tool to map the neurotoxic cascade. There are, however, methodological variations among studies and a lack of consistency in the doses applied, with supra-environmental doses used that are not representative of in situ aquatic hotspots (10-50 µg/L). Future studies should focus on standardization of behavioral testing methods and chronic low-dose exposures to mimic the exposures used in the Ecological Risk Assessment (ERA) framework to increase translational relevance (Figure 1).

Key Words Lead (Pb), *Danio rerio,* Oxidative Stress, Neurotoxicity, Developmental Toxicology, Apoptosis, HPI Axis, Thyroid Dysregulation, Lipid Peroxidation, Epigenetic Programming, Endocrine Disruption, Molecular Biomarkers, Behavioral Phenotyping, Transgenerational Inheritance

INTRODUCTION

Lead (Pb) contamination is a growing threat to the environment worldwide, affecting both past and present industrial and historical sites. Lead is found in multiple forms and in many areas, with informal e-waste recycling activities and the ageing infrastructure being the two most significant sources of exposure that pose a threat to the stability of ecosystems and human health [1]. Although the overall impact on aquatic biodiversity is understood, there is still a great challenge in establishing a clear causal relationship between initial molecular events and complex functional behavioural deficits [2]. Children are especially at

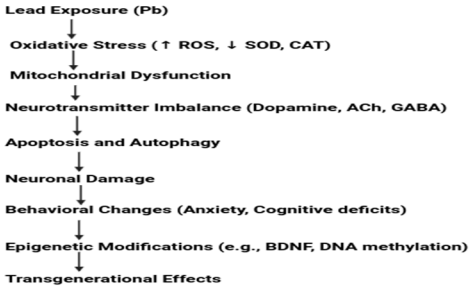

Figure 1: Schematic Representation of the Mechanistic Pathways of Lead (Pb)-Induced Neurotoxicity

risk, as early-life exposure has been associated with a reduction in IQ, a greater occurrence of ADHD and executive functioning deficits [3]. Moreover, chronic exposure to early life is emerging as a risk factor for late-onset neurodegenerative diseases, e.g., Alzheimer's and Parkinson's diseases [4,5]. The effects on the ecology are also remarkable - aquatic animals show neurotoxicity effects of Pb, which include less efficient foraging, less ability to avoid predators and less reproductive success. These individual-level deficiencies can then cascade to population-level changes that can change community structure and function [6]. The zebrafish (*Danio rerio*) is therefore an ideal vertebrate model for neurotoxicological studies, addressing the above challenges. The zebrafish has about 70% genetic similarity to humans and shows strikingly conserved brain development processes, such as the development of the blood-brain barrier and maturation of primary neurotransmitter systems [7]. It is also easy to screen for developmental neurotoxicity in the model, using a non-invasive, high-throughput method, within 24-48 hours after fertilization, by measuring the transparency of the test animals. The model is also suitable for non-invasive, high throughput screening of developmental neurotoxicity in 24-48 hours post fertilization as transparency is measured [8,9].

However, the exposure level in the laboratory is generally at high and acute doses, whereas in the real world, it is in the chronic and sub-lethal range (10-50 µg/L) [10,11] of contaminated environments. This review aims to move from description to critique and synthesis of the narrative. We will systematically follow the pathway from first oxidative stress, ALAD inhibition and second, cell-level failure and attempt to map the mechanisms underlying the observed neurobehavioral phenotype [12]. In conclusion, the review provides a coherent conceptual framework that connects laboratory data with Ecological Risk Assessment (ERA) and No Observed Adverse Effect Levels (NOAEL) and will help the field to move towards more science-related and policy-relevant information [13,14].

METHODS

Search Strategy and Database Parameters

A structured literature search was performed in three main academic databases (PubMed, Scopus, Web of Science) to ensure a more modern overview of the field. The search timeframe was limited to the last 13 years (2013 to 2026) of peer-reviewed research. Boolean search operators and targeted strings were used to search the databases using the following keywords: “lead neurotoxicity”, “zebrafish”, “oxidative stress”, “neurotransmitter”, “behavior” and “epigenetics”. This manuscript, being in the form of a narrative review, used a structured search strategy as a priority to ensure a reproducible and transparent literature selection process.

Study Selection and Evidence Base

A multi-tiered screening process was used to identify relevant, high-impact studies, to build the evidence base. The initial identification by searching the databases generated around 120 unique records. After excluding duplicate studies and an initial screening of titles and abstracts, 70 studies were identified as being relevant to the neurotoxicity of Pb in zebrafish and were evaluated in full text. After thorough consideration, 45 original research papers were picked for final synthesis.

- **Inclusion Criteria:** Only peer-reviewed, English-language papers reporting specific molecular, biomarker, and/or functional behavioral outcomes related to zebrafish model exposure to lead were included
- **Exclusion Criteria:** Studies were excluded if they were not clearly designed as experiments, if they had insufficient experimental controls or if the experiments were not related to neurotoxicity, because they were part of other physiological systems of non-neuronal cells
- **Quality Assessment:** The final selection was determined by the quality of the experimental design, the quality of exposure concentrations reported and the reproducibility of the observed neurotoxic phenotypes

The analyzed literature was synthesized using a qualitative analysis to determine the mechanisms that converged and the major mechanisms of variability. A quantitative meta-analysis was not conducted because the evidence base is methodologically heterogeneous, with varying durations of exposure and endpoints and varying types of behavioral outcomes. However, the synthesis focuses on a critical analysis of the progression of damage caused by Pb from the molecular to the systemic level in the behavioral drift.

Mechanistic Interpretation

Lead (Pb) is a neurotoxic agent that has a complex molecular network with multiple steps, starting with chemical insults and culminating in systemic failures. These stages can be divided into three stages: adaptive response, compensatory failure and terminal neurodegeneration.

Phase I: Adaptive Response and Redox Initiation

Adaptive Response and Redox Initiation: The main effect of Pb on neurotoxicity is the disruption of redox homeostasis. The lead ions (Pb2+) have a high affinity with sulfhydryl groups, which allows them to bind to the essential cations such as calcium (Ca2+) and disrupt the mitochondrial electron transport at Complexes II and III [15]. This mimicry causes the inhibition of δ-aminolaevulinic acid dehydratase (ALAD), which leads to the accumulation of δ-ALA, giving rise to the production of superoxide radicals (Figure 2). First, the organism tries to adapt to it by inducing the Nrf2-Keap1 pathway, leading to the induction of cytoprotective genes such as Heme Oxygenase-1 (HO-1) and NQO1 [11].

Phase II: Compensatory Failure and Neurotransmitter Dysregulation

With prolonged exposure, the antioxidant defence system is depleted. Deficiency of antioxidative enzymes like SOD, CAT and GPx, causes uncontrolled oxidative stress and

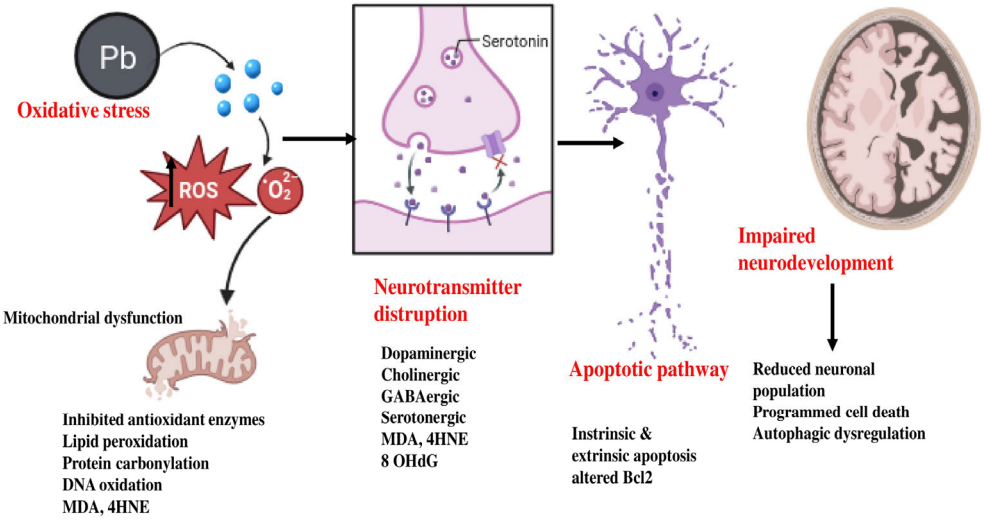

Figure 2: Pb-Induced Redox Stress Triggers a Hierarchy of Mitochondrial Failure and Neurotransmitter Dysregulation, Driving Caspase-Dependent Apoptosis and Permanent Neurodevelopmental Loss

leads to the production of malondialdehyde (MDA) and 4-HNE. Causing a wide-spread lipid peroxidation and protein carboxylation, these products affect the structural integrity of the brain's neural membranes [16]. This Phase II failure is not only an issue in the brain's wiring, it's also a problem within the brain's architecture. Lead acts within the dopaminergic system by inhibiting the activity of tyrosine hydroxylase and changing the expression of transporters that results in changes in hyperactivity in larvae and hypoactivity in adults [17]. At the same time, the cholinergic system is also affected by the inhibition of the acetylcholinesterase, causing the buildup of acetylcholine and also causing a dysfunction of the neuromuscular system [18]. In addition, Pb2+ activates NMDA receptors, binds to NMDA receptors and inhibits GABAergic inhibitory signaling, leading to hyperexcitability of networks.

Phase III: Irreversible Damage and Neurodegeneration

All these molecular alterations may lead to activation of programmed cell death mechanisms and to the loss of neurodevelopmental milestones [19]. Lead activates intrinsic (mitochondrial) and extrinsic (death receptor) apoptotic pathways, which is characterized by the release of Cytochrome C and activation of the death receptor caspase-9/3 executioner cascade [20]. This is followed by an imbalance of Bcl-2 family proteins and thus a pro-apoptotic environment in the cell. What's more, Pb disrupts autophagic flux, causing the buildup of damaged organelles and cellular waste. In the developing zebrafish, these late effects include suppressed neurogenesis and a decrease in numbers of neurons, which results in smaller brains and changes in their neuronal organization that will last a lifetime [21].

Molecular Diagnostic Biomarkers for Pb Neurotoxicity

Unambiguous molecular markers are essential to connect the dots between Pb exposure and systemic physiological failure. Here, these biomarkers are hierarchically arranged in the order of their appearance in the acute redox imbalance-terminal neurodegeneration - transgenerational epigenetic changes (Table 1).

Early-Phase Redox Biomarkers

Initial molecular changes in response to Pb exposure include upregulation of adaptive genes, such as Heme Oxygenase-1 (HO-1). HO-1 is sensitive to redox imbalance and thus is a "good early-warning" sign for activation of the Nrf2-Keap1 pathway [28]. The increase of Malondialdehyde (MDA) on the other hand is the direct measurement of lipid peroxidation induced damage to the membrane structure, which is the beginning of the onset of disease [13].

Biomarkers of Functional and Structural Decay

When toxicological damage is occurring, markers of synaptic and developmental stability are being focused on. The decrease in NRXN2A and CREB1 provides a direct molecular correlate of the cognitive and sensorimotor defects that are seen in behavioral assays [30]. At the same time, the cellular environment is affected by a pro-apoptotic change, which can be assessed by the ratio of Bax/Bcl-2 and activation of Caspase-3 [31]. These markers are critical to help decide between a transient physiological stress and a permanent neurodevelopmental loss.

Emerging Transgenerational Signatures

New zebrafish research uncovered a new class of biomarkers to follow up ancestral exposure through an unexposed F2 and F3 generation. Another epigenetic architecture, DNMT3bb.1 and HDAC4, is dysregulated, causing a molecular signature of exposure to lead that persists [32]. These markers, together with the changes in the expression of neurodevelopmental genes, such as BDNF and GRP, offer a strategic perspective to evaluate long-term and inherited risks. The multi-tiered

Table 1: Hierarchical Molecular and Cellular Biomarkers in Zebrafish

Category	Biomarker	Biological Role	Change after Pb Exposure	Method of Assessment
Neurotransmission [16,27]	Tyrosine hydroxylase (TH)	Controls dopamine synthesis	Reduced expression	Immunohistochemistry (IHC), Western blot (WB), quantitative polymerase chain reaction (qPCR)
	Acetylcholinesterase (AChE)	Regulates acetylcholine levels	Decrease (inhibition)	Ellman's Assay
Oxidative stress [13,28]	Heme Oxygenase-1 (HO-1)/HMOX1	Heme breakdown; stress response marker	Increase (powerful early signal)	Immunohistochemistry (IHC), quantitative polymerase chain reaction (qPCR)
	Malondialdehyde (MDA)	Lipid peroxidation Final product	Increase (Indicator of oxidative damage)	Thio barbituric acid reactive substances (TBARS)assay
	Superoxide dismutase (SOD)/Catalase (CAT)	scavenging free radicals	Decrease or equal (lacking response)	free radical Enzymatic assay
Synaptic plasticity [29,30]	Brain-derived protein1 (BDNF)/cAMP response element-binding protein (CREB1)	memory, learning neuronal survival	Decrease	Western blot (WB), quantitative polymerase chain reaction (qPCR)
	Neurexin-2 alpha (NRXN2A)/neurexin	synaptic organization and adhesion	Decrease	Quantitative polymerase chain reaction (qPCR), Whole-mount In Situ Hybridization (WISH)
Apoptosis [20,31]	Ratio of Bcl2-associated protein (BCL2)/ B-cell leukaemia/lymphoma 2(BCL2)	Modulate mitochondrial apoptosis	Decrease (Pro-apoptotic shift)	Western blot (WB), quantitative polymerase chain reaction (qPCR)
	Caspase-3 activity	Key executioner protease	Increase	Fluorometric Assay
Epigenetic [27,32]	Histone deacetylase (HDAC)/DNA methyltransferase (DNMT)	DNA histone deacetylation and methylation	Transformed	Quantitative polymerase chain reaction (qPCR)
	Global DNA methylation	Epigenetic landscape	changed	Enzyme-linked immunosorbent assay (ELISA), Luminometric Methylation (LUMA)

panels can be integrated to get a more predictive and policy-relevant environmental health assessment [10].

Endocrine Disruption and Epigenetic Programming
Dysregulation of the HPI and HPT Axes

The neurotoxic effects of lead (Pb) are far reaching in the teleost endocrine system, in particular disrupting the Hypothalamic-Pituitary-Interrenal (HPI) and Hypothalamic-Pituitary-Thyroid (HPT) axes. Chronic activation of HPI results in systemic glucocorticoid release that is similar to the mammalian stress response and leads to lead exposure [33] (Figure 3). A persistent increase in cortisol is one of the main causes of neural toxicity, causing a loss of dendritic structures in the hippocampus and a loss of stress-recovery phenotypes in adult zebrafish. At the same time, Pb disrupts the HPT axis, such that thyroid hormone (T3/T4) levels can be measured in a decrease in response to Pb exposure. These hormones play critical roles in basic neurodevelopmental functions, such as myelination, neuronal migration and synaptogenesis and their suppression is, therefore, a key pathway for cognitive deficits resulting from exposure to Pb [34]. In addition, Pb2+ interferes with regulation of genes by mimicking important divalent cations. Lead disrupts the ability of transcription factors (e.g., Sp1) to bind to DNA through its replacement of Zn2+ in the "zinc finger" motifs. This molecular mimicry results in the extensive and pervasive gene cluster dysregulation that is critical for the architecture and functional homeostasis of the nervous system.

Epigenetic Dysregulation and Transgenerational Legacies

The zebrafish model also provides evidence of an important aspect of Pb neurotoxicity-its ability to be passed down through generations via epigenetic remodeling. F1, F2 and F3 generations generations have been found to exhibit inherited behavioral phenotypes even if they have not been directly exposed to the contaminants. They are due to changes in the methylation of the DNA, changes in the histones and differential expression of non-coding RNAs, these being called “epigenetic legacies”. Importantly, genes of great importance to neuroplasticity, such as BDNF and the glucocorticoid receptor, are consistently turned off by these mechanisms. This epigenetic signature is associated with inter-generational anxiety-like behaviours and memory impairment [35]. These results indicate that effects of Pb exposure are not just on the brain of the individual but leave behind a signature of neurodevelopmental risk that lasts beyond the current exposure. The incorporation of these transgenerational perspectives is key for a more integrated approach to global risk assessment for ecology and public health [10].

Behavioral and Cognitive Outcomes: An Ontogenetic Shift

The ontogenetic reversal of zebrafish Pb-induced neurotoxicity is a period from hyperactivity to adult anxiety and hypoactivity. Zebrafish show a specific ontogenetic reversal of neurotoxicity caused by Pb: Hyperactivity and

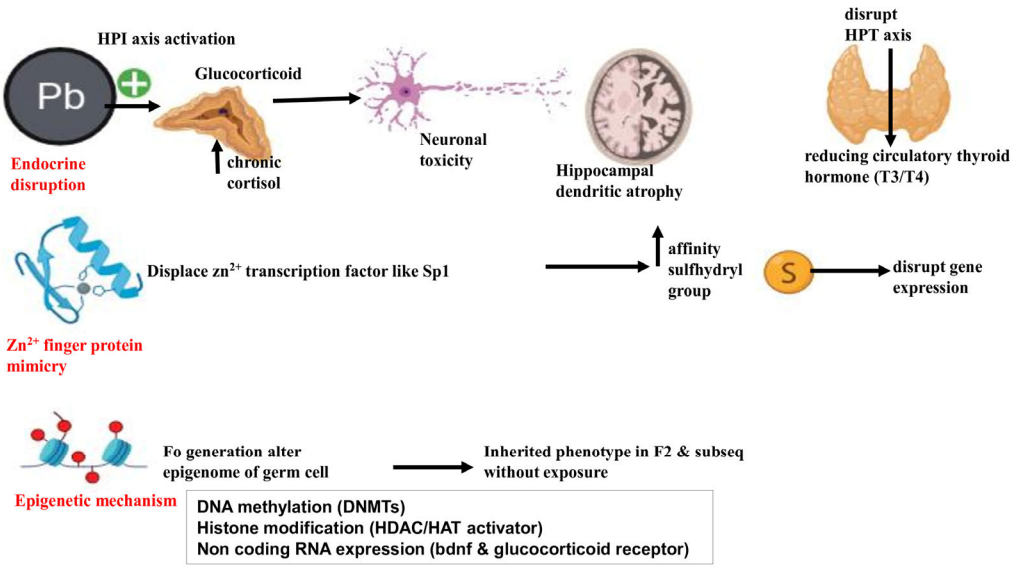

Figure 3: Lead-Induced HPI/HPT Axis Failure and Hippocampal Atrophy, Coupled with Zn2+ Displacement in Transcription Factors, Drive Transgenerational Neurotoxic Phenotypes Through Heritable DNA Methylation and Histone Remodeling

Table 2: Dose-Response Relationships and Evidence Strength

Pb Dosage levels	Exposure Duration	Life phase	Neurotoxic outcomes	Strength of Evidence
Low (≤10 µg/L) [1,2,5]	Chronic	Larvae/Adult	oxidative stress, Subtle behavioral changes	Moderate
Moderate (10-100 µg/L) [3,7]	Acute/Chronic	Larvae	Enzyme changes, Hyperactivity	High
High (>100 µg/L) [6,15]	Acute	Larvae/Adult	Neuronal damage, Apoptosis, hypoactivity	High
Chronic low-dose [27,35]	Long-term	Multi-generation	behavioral deficits, Epigenetic effects	Emerging

then anxiety/hypoactivity in adulthood. At the larval stage (24-120 hpf), exposure leads to large increases in hyperactivity and spatial displacement, which is mechanistically associated with hyperexcitability of neurons. This excitation is brought about by a "dual-hit" to the development of the nervous system, the disruption of the GABAergic system-the brain's chief inhibitory circuit-and the immaturity of the blood-brain barrier [22]. During the maturation process this phenotype becomes hypoactive and anxious. During adult Novel Tank Tests (NTT), this is seen as an increase in bottom-dwelling (thigmotaxis), which involves transition from simple locomotor excitation to complex stress reactions through the Hypothalamic-Pituitary-Interrenal (HPI) axis, the teleost functional homolog of the mammalian HPA axis [23].

In addition to the baseline movement, the effects of Pb exposure also affect higher order sensorimotor and cognitive functions. The lesion of the Mauthner cell-mediated startle response may be associated with an increase in escape latencies and a decrease in habituation, which reflect the defects in rapid neural processing [24]. Cognitive deficits, at the level of the brain, are localized to the dorsolateral pallium (the homolog of the hippocampus) and are associated with a decreased memory retention. These failures are linked to a biological mechanism of inhibiting NMDA receptor signalling and CREB phosphorylation, which are molecular markers crucial for the consolidation of memory [25]. But these behaviour findings have not been easy to replicate. Lab-to-lab variations result from different ambient lighting protocols, automated tracking algorithm sensitivities and variation in water chemistry. Moreover, the use of high, acute exposures in the past has not considered the "behavioral drifts" that result from chronic (Table 2) environmentally realistic exposure levels (10-50 µg/L) [1,22]. The field should emphasize standard designs of assays to better emulate the chemical complexity of human and ecological risk to enhance the models' translational value.

Summary of Pb exposure levels and associated neurotoxic outcomes in zebrafish (Table 2). The table presents general trends observed across studies, illustrating the relationship between dosage level, exposure duration, life phase and neurotoxic outcomes.

Across studies, behavioral outcomes consistently show a developmental shift from larval hyperactivity to adult hypoactivity and anxiety-like behavior, reflecting underlying alterations in neural circuitry and neurotransmission (Table 3).

The Interplay Between Lead and Other Environmental Contaminants

Lead (Pb) works synergistically with co-occurring contaminants in the natural environment and the neurotoxicity of lead is largely altered by these interactions. This interaction involves a shared inflammatory cascade resulting in a rewired cellular environment. The presence of a secondary stimulus, such as an organophosphate pesticide or an organic pollutant, makes microglia more susceptible to

Table 3: Behavioral Assay Frameworks for Pb Neurotoxicity

Behavioral framework	Developmental stage	Parameters Measured	Neurological function Assessment	Pb-induced effect
Light-Dark locomotor assay [30]	Larvae 5-7 days post-fertilization (dpf)/48-70 hours post-fertilization (hpf)	Total distance travelled, locomotor activity, swimming velocity	Basic motor function, visual evoked response, stress reactivity	Larvae: hyperactivity; Adults: hypoactivity
Novel tank diving test [31]	Adult zebrafish ~90 days post fertilization (dpf)	Time spent in top vs bottom zones, latency to enter top zone, immobility episodes	anxiety-like behavior, exploratory activity	Thigmotaxis/reduced vertical exploration, Delayed top-zone entry, increased immobility
Startle Response Habituation [31]	Larvae 5-7 days post-fertilization (dpf)/48-70 hours post-fertilization (hpf) and adult zebrafish ~90 days post fertilization (dpf)	Response latency, Response freqency, habituation rate	sensory-motor function, neural circuit activity, short-term learning	Delayed response, lowered escape velocity, impaired habituation
Passive Avoidance test [32]	Adult zebrafish ~90 days post fertilization (dpf)	Avoidance latency compartment	Associative (learning and memory)	Reduced latency, Indicating impaired memory retension
T-Maze/Plus-Maze [26]	Adult zebrafish ~90 days post fertilization (dpf)	alternation percentage, time spent in arms/zones	Spatial learning, memory, decision-making	Reduced alternation, impaired navigation, cognitive decline

and expresses a hyper-reactive M1 pro-inflammatory phenotype when Pb is present [3,11]. This overactivation leads to excessive production of cytokines such as tumor necrosis factor (TNF-α) and Interleukin-1β (IL-1β) that cause a "dual-hit" on the central nervous system (CNS). IL-1β is also able to actively disrupt the integrity of the blood brain barrier (BBB) [5,15] while TNF-α is responsible for triggering localized apoptotic pathways. The tighter the leakiness of tight junctions, the more toxins will enter the brain from the rest of the body and the more brain tissue will be lost and the brain becomes more leaky. This inflammatory outburst also has a common mechanism: depletion of common antioxidant defenses. A combination of several toxins exhausts quickly the cellular glutathione (GSH) pool and blocks protective enzymes like superoxide dismutase (SOD), thus exposing the dorsolateral pallium to the unchecked oxidative damage of DNA [13,28]. This cumulative toxic burden weakens the primary defense mechanisms, causing substantial behavioural impairments, from memory deficits to chronic anxiety-like reactions, that are much greater than those seen in single exposures to Pb [1,22]. An understanding of this synergy is the norm for these developmental endpoints and highlights an important gap in the existing "single-substance" regulations. Risk assessment needs to be adapted to a mixtures-based approach that enables assessment of the actual hazard in a chemically complex environment, rather than relying on the traditional approach that underestimates the hazard in such environments [10,34].

Environmental Translation

The translation of zebrafish toxicological data to a results that can be used by policy makers will require a paradigm shift from descriptive laboratory observation to predictive Ecological Risk Assessment (ERA). One big challenge for this change is "environmental fidelity": It is the fact that standard lab conditions do not necessarily mimic highly variable pH, temperature and Dissolved Organic Matter (DOM) found in natural environments. Such variables are not just background noise but have been shown to greatly affect the bioavailability and speciation of lead (Pb2+) and directly impact uptake through the gill and skin membrane of aquatic organisms [5,33]. To help span this translational divide, all experimental data needs to be very carefully compared to known regulatory thresholds, such as the No Observed Adverse Effect Level (NOAEL) [34]. Moreover, the zebrafish model also has the potential to become a powerful tool for global policy, as it can be used to evaluate multiple exposures beyond a single substance, which is reflective of the many contaminants present in urban and industrial water bodies. To ensure that water quality standards are protective of aquatic biodiversity and human health, high resolution molecular data must be related to these real world ecological parameters [10,29].

DISCUSSION

The zebrafish is an ideal model to dissect the mechanism of Pb neurotoxicity in a uniquely transparent model, which

connects early-life redox stress and long-term behavioral morbidity. The changes in dopaminergic and cholinergic signaling from molecular disruption to changes in behavior, as summarized in this review (thigmotaxis and cognitive loss), highlight the high translational value of the model [22,23,26]. These results, however, require an understanding of the "concentration gap" that exists in the toxicological literature at this time, if a balance is to be achieved. High dose acute studies provide clear mechanistic information but cannot necessarily reflect "behavioural drifts" that can occur in real world situations of human exposure in contaminated sites [1,5,29]. Moreover, epigenetic legacies is a paradigm shift in the understanding of environmental effects of lead. Current risk evaluation models that are almost exclusively based on the individual who is directly exposed are also inadequate, as neurobehavioural deficits may be transgenerational [27,35]. The combination of these hereditary risks and synergistic effects that are found in mixture studies may start to combine a "mosaic of molecular data into a coherent, predictive tool" [3,34]. Finally, the future of Pb neurotoxicology will be defined by more analogous laboratory protocols to Ecological Risk Assessment (ERA) parameters and by establishing public health policies based on these findings. [33,35].

Implications for Practice

The zebrafish model offers a scalable and economical tool to make high throughput mechanistic screening coupled with proactive environmental surveillance. Researchers can use molecular biomarkers, in particular HO-1 and MDA, as very sensitive early-warning markers of toxicity to produce high-resolution data that is crucial for rapid toxicity evaluation in urban and industrial waters [5,17]. These molecular readouts can be used in conjunction with functional behavioral assays to translate bench science to evidence-based management strategies [1,22]. Such an integrated approach is essential to the development of sensitive monitoring protocols that will be able to detect sub-lethal neurotoxic risks well before measurable changes in ecology or human health outbreak [34]. In addition, the implementation of standardized zebrafish assays in regulatory testing provides a tool to establish more precise No Observed Adverse Effect Levels (NOAEL), which, in turn, will enable the development of environmental protection standards that are based on predictive, functional data and not only on the observation of adverse events after the fact [10,33].

CONCLUSIONS

This important synthesis shows that in the zebrafish model, the cascade of events that control lead (Pb) neurotoxicity is tightly coupled, comprising the processes of oxidative stress, neurotransmitter dysregulation and programmed apoptotic signaling. Redox biomarkers are strong indicators for early exposure but there are also many challenges and questions about reversing the neurotoxic damage. In particular, knowledge of the mechanisms and long-term consequences of epigenetic changes in early life is necessary to address the mechanisms of “mechanistic persistence” of lead effects in the developing brain. The definition of chronic environmental risk goes beyond simply high dose laboratory studies in a multi-contaminant world. Researchers can ensure that the evidence they generate will be the final policy that will be used in the world for managing lead. Finally, the combination of developmental phenotyping with Ecological Risk Assessment (ERA) parameters will ensure that zebrafish model will be a key tool to safeguard aquatic biodiversity and long-term public health.

Future Recommendations

Future studies and research will need to focus on methodological harmonization as a prerequisite to ensure the zebrafish model has the highest possible level of translatability and reproducibility to other independent research groups [9,10]. A paradigm shift is needed from acute, high dose experimental settings to environmentally credible, chronic, low dose experimental settings. In particular, these concentrations need to be in the range of 10-50 µg/L which reflects the chemical profile of real world aquatic hotspots [1,5]. Moreover, using the complex mixtures perspective (not single-contaminant studies) will be more valid for assessing ecological risk with the help of oxidative and epigenetic biomarkers that are assumed to be ontogenetically stable. Strategic activities that focus on these areas will enable a successful transfer of research activities from the bench to Ecological Risk Assessment (ERA) [34]. Finally these developments will make zebrafish data predictive and be able to guide the development of proactive and evidence-based global policy on lead management [33,35].

Limitations

The translation potential of this synthesis is limited primarily by the intrinsic brain anatomical differences between teleosts and humans and by the current zebrafish literature that is rife with methodological differences [7,9]. There is a high level of experimental noise caused by significant differences amongst the assays and the sensitivity of the automated tracking algorithms in the independent laboratories and the variable time periods of exposure to the assays [23,26]. This variability leads to the difficulty in comparison of results and reduces the possibility for a direct quantitative meta-analysis. In addition, the field is still based on the assumption of acute, high dose exposure, which has led to a large “concentration gap”. Much work has already been done on concentrations far above environmental reality will prevent detection of small but chronic, changes in behaviour that occur at lower concentrations (10-50 µg/L) typical of natural habitats [1,11]. All of these elements highlight the critical need for standardised toxicological procedures and harmonised behavioural endpoints. Standardization is essential if the zebrafish model is to be used in human health risk assessment with the predictive power and reproducibility it promises are to come to fruition. If the zebrafish model is to be used for human health risk assessment with the predictive power and reproducibility it promises, it must be standardized. [10,22].

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