



Review Article

Emerging various environmental threats to brain and overview of surveillance system with zebrafish model

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ABSTRACT

Pathologies related to neurotoxicity represent an important percentage of the diseases that determine the global burden of diseases. Neurotoxicity may be related to the increasing levels of potentially neurotoxic agents that pollute the environment, which generates concern, since agents that affect children may increase the incidence of neurodevelopmental disorders, affecting the quality of life of future citizens. Many environmental contaminants have been detected, and many of them derive from several human activities, including the mining, agriculture, manufacturing, pharmaceutical, beverage and food industries. These problems are more acute in third world countries, where environmental regulations are lax or non-existent. An additional major emerging problem is drug contamination. Periodic monitoring should be performed to identify potential neurotoxic substances using biological tests capable of identifying the risk. In this sense the fish embryo test (FET), which is performed on zebrafish embryos, is a useful, reliable and economical alternative that can be implemented in developing countries.

1. Introduction

Neurotoxicology is a complex field in which diverse disciplines, including neurobiology, neurophysiology, pharmacology, genetics, histopathology and clinical neurology, among others, converge. Its objective is to evaluate the harmful effects of various potentially toxic agents on the central and peripheral nervous systems. The global incidences of neurological and mental health diseases have progressively increased due to various factors, including an increased life expectancy, changes in habits and lifestyles that generate stress, a sedentary lifestyle, the use of psychoactive substances, diverse types of trauma that affect the nervous system, and particularly the increasing environmental contamination due to the increase in the concentrations of classical or new neurotoxic substances in the environment. These factors constitute current and future public health problems, particularly in developing countries. Toxicological tests are required to identify toxic levels and biological effects; thus, zebrafish have emerged as a useful model for identifying toxicological risks. For the present review, a bibliographic search of the PubMed, TOXNET, TOXLINE, EMBASE,

SciELO databases and the Google Scholar search engine was performed using a combination of the terms neurotoxicology, environmental contamination, drugs, zebrafish.

2. Environmental stressors increase the risk of neurotoxicity

The global burden of disease study highlights neurological diseases as one of the greatest threats to public health [1]. Many of these problems are expressed at individual and collective levels as neurological disorders or emotional disorders. These disorders cause different levels of intolerance and aggressiveness, which are symptoms related to violence phenomena, in addition to neurological deficits, disability and mortality. Diseases that are part of the global burden of disease include neurodegenerative syndromes, neurovascular diseases, infectious diseases, and nutritional and traumatic diseases, among others. Some of these disorders may be related to the neurotoxic effects of exposure to pollutants, such as mercury, present in the environment in high concentrations or even in low concentrations, since these pollutants may generate subclinical conditions that cause long-term cognitive

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alterations secondary to their neurotoxic effects [2]. It is estimated that approximately 3% of neurodevelopmental disorders and disabilities are caused by exposure to neurotoxins, which induce neuronal damage; these pathologies include intellectual disability, attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder [3]. According to some experts, the prevalence of childhood diseases, such as asthma, cancer and neurodevelopmental disorders, is increasing and may be related to increasing levels of pollution, but no studies have been performed to support these postulates [4]. However, clinicians generally express concern about the risk that exposure to novel neurotoxic agents poses to the general population in both rural and urban areas. For this reason, the WHO has developed a program related to environmental influences on childhood diseases (ECHO) as part of the global environmental health (GEH) program.

In rural areas, the increasing implementation of diverse industrial activities generates environmental pollution and a potential risk of neurotoxicity. For example, intensive and extensive agricultural exploitation generates new risks because it uses a significant number of herbicides, pesticides, fertilizers, and hormones for germination and growth. In addition, mining activity, both legal and illegal, damages ecosystems and generates enormous levels of environmental contamination with various products, including heavy metals and various organic agents [5–9]. These diverse activities share a common element, the use and discharge of various chemical agents to the environment, which increases the likelihood that humans will be exposed to multiple new neurotoxic substances [10–12]. These substances include heavy metals, such as lead, iron, and aluminum, organic compounds, such as pesticides and herbicides, and inorganic substances, such as arsenic salts and mercury salts [13–15,7]. Although international conventions and agreements (Minamata, Stockholm and Basel conventions) exist to control and manage the use of these toxins, many countries have not adhered to these agreements and other countries are in the process of implementing them. Thus, if contamination exists in a country or area, pollution can affect all countries in a specific region, which has been observed for contaminants such as Hg that are emitted in a specific area and contaminate many countries.

In urban areas, accelerated housing construction and densification, both in small and large cities with careless environmental standards, favors urban pollution of all kinds, but also favors environmental deterioration due to the poor disposal of debris and waste, which is associated with the destruction of ecosystems and the disruption of the natural equilibrium. The massive increase in social activities in cities that include public shows and night activities of various kinds have led to changes in consumption habits and favor or stimulate the intake of abuse substances, such as alcohol, tobacco, and hallucinogens, with a risk of acute or chronic toxicity [16]. These substances represent an increasing serious public health problem, due to the increasing consumption of hallucinogens driven by illegal drug trade agents who are searching for new local markets [17,18]. However, public health problems include not only alcoholism, smoking, or addiction to psychotropic drugs but also safety issues arising from the use of psychoactive drugs for criminal purposes (robbery and kidnapping) and the risk of neurotoxicity and death of the victim.

The topic of the toxicity of psychotropic substances also reveals another potentially important factor contributing to environmental contamination: drugs. The problem is linked to the medicalization of society, which is reflected in the tendency to consume medicines for all conditions and the prescription and use of multiple drugs simultaneously to promote both human and animal health [19–21].

The neurotoxicity of the drugs is triggered by a direct action, side effects, or accidental consumption of toxic doses, which can cause neurological disorders at any stage of life. The neurotoxic effect is observed as different types of malformations during development and disorders in psychomotor development, which have dire consequences for the future of the exposed individual due to the vulnerability of the nervous system in prenatal and early postnatal stages. In the adult

individual, neurotoxicity also causes dysfunctional alterations or neurological damage in the mature nervous system. Drugs with potential neurotoxic effects may belong to categories as diverse as anticonvulsant drugs, anxiolytics, antihypertensive drugs, antibiotics, and particularly steroid hormones that are currently considered an increasing proportion of environmental pollutants [22,23]. The use of these drugs is not exclusively limited to humans; the agricultural industry has used large amounts of hormones and antibiotics to guarantee the industrial production of animals and plants. Drugs consumed by living organisms are eliminated either in an unaltered form or as metabolites and will be collected in domestic or institutional wastewater, which eventually contaminates rivers and lakes and constitute the first form of environmental contamination by drugs. On the other hand, the drugs that are not consumed are generally not disposed of in an adequate and proper manner and instead are commonly placed in trash containers from which they are dispersed into the environment. This contamination affects people and wildlife, mostly in developing countries. For example, nonsteroidal anti-inflammatory drugs (NSAID) contamination caused an almost complete extinction of many species of vultures in India [24,25].

All these problems, which are associated with the inadequate disposal of unused drugs or waste products and their metabolites, suggest that drugs are a new and additional element of contamination with potential neurotoxic effects [26–30]. This form of contamination may explain the increasing incidence of neurological disorders in the current population and suggest the possibility that the incidence of neurological disorders will be substantially increased in future generations, because individuals will be exposed to various substances during the prenatal period and will experience a more contaminated planet during the postnatal period. All these factors increase the likelihood that an individual will suffer from various neurological disorders, including intellectual disabilities, motor disorders, hearing disorders, vision disorders, depression, bipolar disorder, autism spectrum disorder, and psychotic disorders, among others [31,32].

In many countries of Latin America and the Caribbean, such as Colombia, Peru, Chile, Ecuador, and Bolivia, among others, the problems of contamination and neurotoxicity are concerning, given the tendency of the economy to shift towards agroindustry development, intensive livestock exploitation, and legal and illegal mining (Fig. 1). In addition, various infrastructure projects are currently being developed that will disrupt the environment, contaminating water, air and land sources with wastes of different categories. These factors represent a series of risks related to neurotoxicity that are not sufficiently explored and may represent a public health risk.

3. Risk management of Environmental pollutants as Neurotoxic substance

Basic research, clinical research and reports of clinical effects have improved our knowledge of the neurotoxic effects of substances such as lead, mercury, arsenic and toluene, which in turn led to the development of evidence-based prevention programs. Unfortunately, the numbers of substances that appear as environmental pollutants have increased, and unfortunately, the information about these pollutants is limited and insufficient to support the development of comparable prevention and intervention programs.

Currently, 70,000 chemicals are considered potentially neurotoxic, but the effects have been identified for only 10% of these chemicals. In the occupational health field, approximately 40% of new diagnoses are believed to be related to these substances. The existing concern has precipitated a research program led by a global health organization in the field of global environmental health (GEH), in which diseases related to effects of toxic substances on neurodevelopment represent an important line of research (Table 1).



Fig. 1. Mining and environmental impacts. The satellite image shows areas of open-pit gold mining in the municipality of Zaragoza, Colombia. The box shows the proximity of the exploitation areas with the Nechí River. The Remedios and Segovia sectors in Colombia are considered the zones with the highest levels of mercury contamination in the world. (Taken from Google maps 7° 29'21.5" N 74° 52'13.4" W).

4. Neurotoxic environmental factors

Environmental factors that potentially generate neurotoxic effects are organized into four broad groups including:

Agents related to environmental pollution, including radiation, heavy metals, organic substances, drugs and other substances present in air and water [33,34].

Agents related to climate change, including earthquakes, tsunamis, volcanic seismic activity that produce radiation, heavy metals and other substances present in air and water [35].

Agents related to the occupational environment: radiation, organic substances, drugs, heavy metals [36].

Agents related to the use of addictive substances: alcohol, tobacco, amphetamines, heroin, and cocaine [37].

5. Routes of exposure

The routes of exposure are diverse and include the digestive tract, airways and skin. Exposure can occur in massive and acute doses, by overdose of a single dose or by the chronic administration of low doses. Moreover, neurotoxic substances may affect an individual or an entire community. The most vulnerable populations include the prenatal and infant populations, because their nervous systems are developing, and the population of older adults, because they may exhibit neurological deterioration that is potentially accelerated in the presence of different levels of neurotoxic substances in the environment.

6. Optimization model to study Neurotoxicity with Environmental pollutants

When neurotoxic agents are suspected to be present in an individual or in a community, the potential toxicant must be suspected, identified and isolated in the environment through monitoring. Subsequently, both preclinical tests and clinical evaluations are required to confirm suspected neurotoxicity caused by a pollutant. Preclinical methods used to perform these evaluations include cellular and systemic studies. The methods used for these evaluations include biochemical, histopathological, electrophysiological, behavioral, and pharmacological methods. From a clinical point of view, specific signs of toxicity are frequently difficult to detect, and the manifestations can be confused with other neurological disorders, ranging from stress to specific diseases of the nervous system. Therefore, the diagnosis is only confirmed by obtaining an exposure history and identifying the toxic substance in the organism [38].

Upon exposure to low concentrations of a potentially neurotoxic agent, the initial number of people affected is generally low. As the concentration increases over time, the first cases of toxicity begin to appear in adults. Afterwards, the subclinical effects on the infant population and the first cases of adult poisoning begin to appear. These cases may reflect a silent pandemic problem, which increases the disease burden, increases the cost of the health care and increases the inequity and poverty in many regions of developing countries.

7. Environmental monitoring

Researchers must evaluate the presence of neurotoxic substances and their levels in the affected area. This monitoring includes environmental measurements obtained from the air, water, foods and soil and biological monitoring of exposed individuals, which includes analyzing the levels of substances present in the blood, secretions and exhaled air. The primary chemicals or their metabolites, which are known as exposure biomarkers, are measured using this process. The effects of the substances on specific targets, such as enzymes and proteins, are also measured. These targets are called biomarkers of effect, as hemoglobin, albumin, and transaminases. Finally, biomarkers of susceptibility assist in detecting individuals or populations that are more susceptible to toxic effects, such as the case of slow acetylators.

8. Neurotoxicity studies in biological models

The biological models used to assess neurotoxic substances include *in vitro* and *in vivo* models and range from subcellular structures to whole animals. Subcellular organelles include membranes and organelles; individual cells include neurons, epithelial cells and myocytes. Isolated organs include the heart, lung or kidneys and whole organisms include invertebrates and vertebrates, such as insects, mollusks, fish, amphibians, rodents, and primates.

The use of these models is vitally important to the study of the acute, subacute and chronic effects of neurotoxic substances. These models are also important for identifying potential toxicity in prenatal, postnatal and transgenerational stages, since various substances can accumulate and generate damage in both the exposed individual and their descendants [38].

9. Zebrafish model for studying environmental factors-driven neurotoxicity

Although an increasing number of environmental pollutants has

Table 1
Substances associated with neurotoxic damage.

Toxic agent	Pathologies	Source
Metals		
Arsenic (As)	Acute encephalopathy Peripheral neuropathy	Pesticides Seafood Semiconductors
Lead (Pb)	Encephalopathy Peripheral neuropathy	Welding services Lead bullets Illicit whiskey Insecticides Batteries Paintings
Manganese (Mn)	Encephalopathy Parkinsonism	Iron industry Fertilizers Manufacture of dry batteries
Mercury (Hg)	Acute: headache, nausea, and tremor Chronic: ataxia, peripheral neuropathy and encephalopathy	Scientific instruments Dental amalgams Electroplating industry Photography Mining
Copper (Cu)	Acute: memory defects, convulsions, and disorientation Chronic: encephalomyelopathy	Welding Electronic components Polyvinyl chloride Fungicides
Solvents		
Carbon Disulfide	Acute: encephalopathy Chronic: peripheral neuropathy	Preservatives Textiles Varnishes Electroplating industry Paintings
Methyl <i>n</i> -hexane	Narcosis Peripheral neuropathy	
<i>N</i> -butyl ketone	Peripheral neuropathy Narcosis	Lacquers Varnishes Paint removers Glue Adhesives
Tetrachloroethylene	Acute: Narcosis Chronic: peripheral neuropathy and encephalopathy	Paint removers, degreasers, extraction substances, and the textile industry
Toluene	Acute: Narcosis Chronic: encephalopathy and ataxia	Glue Manufacture of benzene Gasoline, aviation fuel, paints
Trichlorethylene	Acute: Narcosis Chronic: encephalopathy and cranial neuropathy	Degreasers Painting industry Varnishes Dry cleaning industry
Insecticides		
Organophosphates	Acute: cholinergic poisoning Chronic: ataxia, paralysis, and peripheral neuropathy	Agriculture industry
Carbamates	Acute: cholinergic syndrome Chronic: peripheral neuropathy and tremor	Agriculture industry
Gases		
Carbon monoxide	Acute: headache, dizziness, nausea, impaired cognitive functions, and loss of consciousness Chronic: Parkinsonism after a period of pseudo-recovery	Incomplete combustion of organic materials such as gasoline and gases (methane and propane)
Ethylene oxide	Acute: respiratory tract irritation, nausea, headache, and vertigo Chronic: peripheral neuropathy	Sterilization units in hospitals Medical equipment industry
Drugs		
Thalidomide	Deafness	Maternal ingestion and environmental contamination
Methotrexate	Microcephaly and myelomeningocele	Maternal ingestion and environmental contamination
Trimethoprim	Neural tube defects	Maternal ingestion, environmental contamination, and foods
Phenytoin	Neural tube defects	Maternal ingestion and environmental contamination
Alprazolam	Abstinence syndrome	Maternal ingestion and environmental contamination
Atorvastatin	Myopathies	Maternal ingestion and environmental contamination

been reported and basic research of these factors is required, paradoxically, basic biomedical research is subject to an increasing number of restrictions each year, which reduce the possibility of establishing cause and effect relationships between environmental exposure and neurotoxicity. Limitations and restrictions include the high cost of animal models and bioethical issues regarding the use of mammals for toxicological studies. For this reason, models of non-mammalian vertebrates with genetic similarities to humans have been utilized as alternative animal models, and zebrafish has progressively become one of the most frequently used models. A Google Scholar search shows a

progressive increase in the number of publications related to neurotoxicology from 10 articles published between 1995–1999; 2680 articles in the period 2010–2015. The combination of the terms zebrafish, neurotoxicology and contamination resulted in 6080 total publications and 1130 publications since 2016. Thus, the issue of pollution and its impact on the nervous system has begun to be addressed in this model so, we consider that this field has a lot of opportunities for researchers (Fig. 2).

Interest in employing zebrafish as a model in biomedical basic research has increased due to several key features, including their high

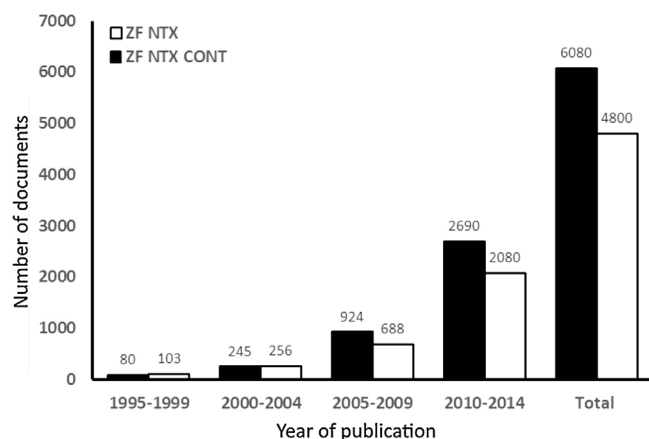


Fig. 2. Numbers of papers published during 1995–2015 years. Search was performed with keywords “zebrafish and neurotoxicology”; gray bars and “zebrafish neurotoxicology and contamination”; white bars.

reproductive capacity, small size, external fertilization and embryonic development, rapid development and short life cycle. In addition, the embryo and larvae are transparent in the first weeks of life, and the zebrafish genome has recently been sequenced [39]. Another important aspect to consider is the administration of water-soluble drugs or chemicals, which is simple and amenable to large-scale mutagenesis studies. Currently, protocols for care and reproduction are clearly defined [40] and extensive documentation on bioethical standards to be followed in experimental studies is available [41,42]. Another advantage is the large amount of information on zebrafish available online, and databases related to genetic resources and various useful protocols and techniques for researchers have been reported. The University of Oregon has centralized information on this model and offers wild populations, as well as mutant and transgenic lines [43]. Finally, zebrafish populations are easy maintained at relatively low cost in the laboratory. The need for animal handling is minimal, which also reduces the requirement for technical staff. The size of adult fish is normally less than 5 cm in length which facilitates the care and maintenance of many specimens in a small space. The costs associated with zebrafish facilities and maintenance are low compared with the costs for rodent laboratory animal housing. In addition, some authors have developed custom-made zebrafish housing at low cost which could be useful to investigators with low financial support for research [44,45].

Several studies have now been conducted on zebrafish exploring the effects of various potential environmental contaminants, including methylmercury [46,47], uranium [48], cadmium [49–51], methylparathion [52,53] and benzopyrenes [54,55] on different structures and organs, particularly on nervous system. Heavy metals and industrial chemicals are known causes of neurodevelopmental disorders and subclinical brain dysfunction and these studies in zebrafish embryos and larvae support the hypothesis that exposure to these chemicals during early fetal development can cause brain injury at quantities lower than those affecting adult brain function. This information allows us to affirm that the zebrafish model is a powerful tool for the study of environmental contaminants and their effects on the nervous system. Recently, an in vitro method (fish embryo test, FET) has been reported and presented as a tool to determine the acute toxicity of wastewater containing possible pharmaceutical contaminants [56,57]. FET is a simple, rapid and cost-effective test. Some advantages of this model include the possibility to have the embryos available permanently; only small amounts of test substance or wastewater are required and the effects can be evaluated from a molecular, genetic, functional and developmental point of view [58]. Therefore, we consider that FET could be used as an accessible and inexpensive biological test to evaluated adverse effects of environmental chemicals on nervous system.

10. Perspective of neurotoxic assessment and limitation

Neurotoxicology constitutes a large field of work and study in developing countries, with multiple topics that include the relationships with environmental pollution, occupational health, addictions, nervous system development and function, and neuronal diseases. Approximately 3% of alterations in neurodevelopment presenting as intellectual disabilities are estimated to be a consequence of exposure to toxins, whereas 25% of these alterations are derived from the interaction between toxic substances and genetic susceptibility, representing part of the global burden of disease.

Many of the toxic effects of various substances not only affect the individual but also the entire community. Toxicity may have a substantial impact on the public health field, given the costs of these diseases due to mortality and particularly disability. The educational field is also affected, which is reflected in results related to school performance, school dropout, educational level and number of competitive professionals [59]. These results in turn affect economic sectors due to the low productivity related to disabled professionals, labor incapacities, treatment costs and rehabilitation. Finally, the rates of social problems, including poverty, marginalization, inequality, insecurity and high crime rates, may increase.

The degree of contamination and the type of contaminants must be identified to generate a risk map with the levels of contaminants and exposure and their impacts on the health of the population and to propose solutions to neurotoxicity caused by increasing pollution levels. Contaminant sources must also be monitored to permit the development of clear regulations that coerce toxic waste generators to control or eliminate emissions [60,31,61,5].

However, multiple stakeholders limit or prevent the implementation of regulatory mechanisms that prohibit or restrict the use of potential neurotoxic contaminants. On one hand, several international companies are interested in promoting the consumption of chemical products, such as the agrochemical industry, the food and beverage industry, the pharmaceutical industry and the illegal drug trade. On the other hand, industries may be interested in avoiding any regulation, since they restrict the use of chemicals and therefore threaten profitability. These industries include the agricultural industry, the legal and illegal mining industry, the petrochemical industry, the textile industry, the automotive industry, the electronics industry and the pharmaceutical industry.

Disclosures

We declare that we do not have any conflicts of interest in relation to this article. No ethical issues are involved.

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