Rev. Biol. Biomed. Sci. 2022-2023 5 (1) 19-28
DOI: 10.31178/rbbs.2022-2023.5.1.3

Fish larvae: an alternative animal model for testing the toxicity of food additives

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Received: 6 December 2023 / **Revised**: 10 January 2024 / **Accepted**: 11 January 2024 / **Available online**: 12 January 2024

Abstract Concerns regarding the properties and potential hazardous effects of food additives are increasing. Food additives are widely used because they improve the taste, texture, color and shelf life of food. While commonly consumed worldwide, food additives have begun to raise more concerns regarding their impact on human health and the environment. Thus, new extensive but animal-minimized testing strategies are needed to highlight the short- and long-term effects of the many food additives. Thus, it was necessary to find alternatives for toxicology studies, such as *ex vivo*/*in vitro* testing, embryos and larvae that have external fertilization and external embryonic or larval development. There are many studies that highlight the usefulness of non-mammalian models, such as fish embryos and larvae. Fish embryos and larvae are preferred for *in vivo* studies because they have numerous advantages, such as external and short-term development, embryos and larvae are transparent, and genes, receptors and molecular processes are highly conserved between human and fish. In this review article we aimed to highlight the studies that tested the toxic effects of food additives on fish larvae, the toxicological parameters necessary to establish toxicity and the most common anomalies in fish larvae caused by food additives.

Keywords: food additives, fish larvae, larval body malformations, edema, spinal anomalies

Introduction

Food additives are widely used to improve the taste, color, aroma, texture and extend the shelf life of food or beverages (Martynov and Brydyrenko, 2018). Additives are divided into natural and synthetic additives, and based on their functions, food additives are divided into other groups, such as colorants, antioxidants, preservatives, thickeners, bleach etc. Although food additives offer numerous benefits, excessive and improper use is a threat to the health of the population (Wu et al., 2021).

The toxicity of chemicals, including food additives and dyes, is initially evaluated on model organisms (Busch et al., 2011). Model organisms allow the study and understanding of specific biological processes, thus providing useful and essential information that can be extrapolated to other organisms, including humans (Carnovali et al., 2019; Khan and Alhewairini, 2019). The European Union, through Directive 86/609/ECC (European Commission of 1986), followed by Directive 2010/63/EU (European Commission of 2010) restricted the use of higher vertebrates in research. Thus, it was necessary to find alternatives for toxicology studies (Stelzer et al., 2018), such as *ex vivo/in vitro* testing, embryos and larvae that have external fertilization and external embryonic or larval development (Falcão et al., 2018; Vranic et al., 2019). There are numerous studies in the literature that highlight the usefulness of non-mammalian models, such as fish embryos and larvae (Busch et al., 2011; Brannen et al., 2016).

In the early stages of development of most fish species, embryos and larvae are transparent, which simplifies direct observations of organogenesis without the need for additional invasive procedures (Genest, 2019). Also, in oviparous species, fertilization and development are external, thus the effects of food additives can be evaluated *in vivo* (Spitsbergen and Kent, 2003).

Danio rerio is among the fish species that are widely used in biological development and for studying organogenesis in vertebrates (Genest, 2019). Most of the experiments that evaluated the toxicity of food dyes and additives were performed on *Danio rerio*. Fish, such as *Danio rerio*, present numerous advantages that have determined their use as model organisms, such as external and short-term development (Khan and Alhewairini, 2019), embryos and larvae are transparent, and genes, receptors and molecular processes are highly conserved between *Homo sapiens* and *Danio rerio*. *Danio rerio* shows a high level of homology with humans, presenting many similarities at the level of the brain, digestive tract, musculature, vascular system and immune system (Carnovali et al., 2019). Also, fish embryos and larvae are ideal organisms for testing food

compounds because they are permeable to small molecules during organogenesis (Bailone et al., 2022).
Many studies based on the classification of teratogenic and non-teratogenic substances have demonstrated a high correlation of toxic response between fish and mammals, of over 72%. Therefore, fish embryo and larvae are considered one of the relevant, reliable, controllable and reproducible models for studying the impact that various chemical substances have on the human body (Genest, 2019; Alves et al., 2021).
Recently, numerous toxicology studies on fish embryos and larvae have been published, including studies testing the effects of food additives. Table 1 lists the studies that tested the effects of food additives on fish larvae, as well as their effects.

The morphological anomalies of the larvae

Fish larvae have many critical periods in their development, which can be affected by the environmental factors, leading to phenotypic response. Morphological anomalies and behavioral changes occur during the ontogenetic development of fish depending on exogenous factors and physiological characteristics that affect the metabolism of each specimen (Martinez-Leiva et al., 2023). In general, fish larvae anomalies are caused by environmental contaminants, nutrient deficiency, oxygen deficiency, sudden changes in temperature, mutations, parasitic infections etc. (Sajeevan and Anna-Mercy, 2016).
Morphological anomalies are a direct indicator of the toxic potential of chemical compounds, including food additives. The morphological anomalies reported in the literature following the toxicity testing of food additives on fish larvae can be divided into macroscopic anomalies and microscopic anomalies.

Macroscopic anomalies

Studies testing the toxicity of food additives on fish larvae have mostly reported macroscopic anomalies. Table 1 lists the abnormal changes induced by food additives on fish larvae. The most reported macroscopic anomalies are spinal anomalies, yolk sac anomalies and cardiac anomalies.

1. Spinal and muscle anomalies

Spinal anomalies have been reported in all fish species studied and can be observed early in development (Boglione et al., 2013). These defects can be observed from the embryonic period, but become macroscopically evident after hatching (Behra et al., 2002; Joshi et al., 2018; Jiang et al., 2020; Kiziltan et al., 2022). The most common spinal anomalies are in the form of curvature, dislocation, shortening and twisting. Some severe cases are associated with macroscopic deviations of the vertebral axis and include lordosis (V-shaped dorsal-ventral curvature), kyphosis (Λ-shaped dorsal-ventral curvature), and scoliosis (lateral curvature) (Boglione et al., 2013) and determine the abnormal appearance of the body (Kerniske et al., 2021) (Fig. 1).

Fig 1. *Hypophthalmichthys molitrix* larvae. **A**: normally developed larvae 48 hours post fertilization. **B:** abnormal larvae 72 hours post fertilization with spinal scoliosis (arrow) and cardiac edema (arrowhead). Ristea and Zarnescu; unpublished observations.

Spinal anomalies are often reported in the literature after testing different food additives on fish larvae. These anomalies can be explained by the fact that food additives can influence neuronal and muscle acetylcholinesterase (AchE), acetylcholine (Ach) and its receptor systems because AchE and Ach are required for neuronal and muscular development in the zebrafish embryo (Behra et al., 2002; Joshi et al., 2018; Jiang et al., 2020; Kiziltan et al., 2022). Also, under the influence of certain food additives, such azo dyes, it has been reported that neuroinflammatory signals in the cerebrospinal fluid may be involved in affecting spinal nerve function (Jiang et al., 2020).
Another malformation is the incomplete development of the tail. During the tail-bud stage the tail begins to separate from the yolk sac and continues to elongate. Abnormalities during development are also influenced by changes in osmotic pressure in the environment because changes in osmolality can affect the metabolic activities of cells (Li et al., 2023). Li et al. (2023) reported that the longer the exposure time, the shorter the tail.
Weigt et al. (2012) reported that spinal malformations observed in zebrafish embryos and larvae correspond in part to central nervous system abnormalities found in human embryos that have warfarin syndrome. Also, spinal anomalies have been reported to occur due to reduced myosin and myotom formation that are required for normal skeletal muscle development (Liu et al. 2017).

2. Yolk sac anomalies

The yolk sac contains proteins, lipids, and micronutrients that support metabolic function and growth before embryos and larvae begin feeding (Hagedorn et al., 1998). Lipophilic xenobiotics from the surrounding aquatic environment can be selectively aggregated in the yolk sac and can alter the rate of absorption of nutrients from the yolk sac. Fish embryos and larvae have a water barrier that maintains an osmotic gradient compared to the surrounding aquatic environment. Since the yolk sac has a low permeability to water, toxic substances can

affect the osmotic gradient. Therefore, there is an excessive accumulation of water in the yolk sac, which causes the appearance of edema (Sant et al., 2018, Jiang et al., 2020; Merola et al., 2020). Also, yolk sac malabsorption has been listed as a factor associated with microphthalmia, among other factors such as developmental delay or corneal and retinal defects (Duy-Thanh et al., 2023).

Abdominal cavities caused by yolk sac edema are a common feature of fish exposed to toxic substances and it is thought to be caused by overhydration of the yolk sac in association with dysregulation of osmotic homeostasis and toxin accumulation (Merola et al., 2020).

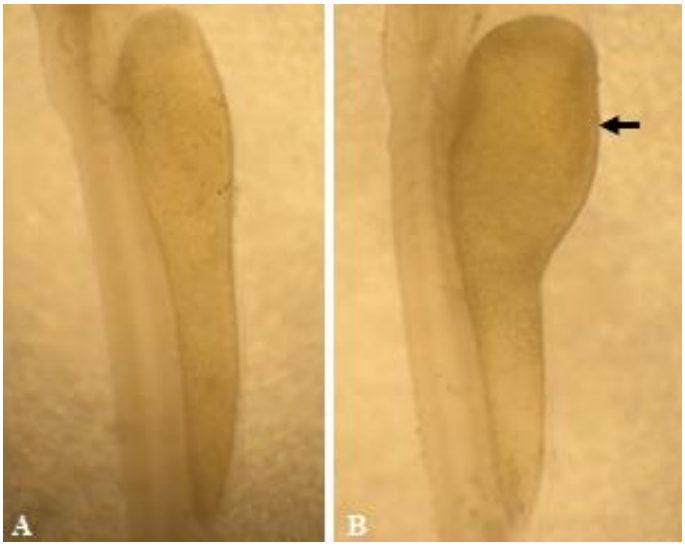

Fig 2. *Hypophthalmichthys molitrix* larvae 48 hours post fertilization. **A**: normally developed larvae. **B**: larvae with yolk sac edema (arrow). Ristea and Zarnescu; unpublished observations.

3. Cardiotoxicity and cardiac anomalies

The heart is one of the first organs that form during development in fish, so measuring heart rate is essential to determine the developmental toxic effect of a xenobiotic (Li et al., 2017).

During normal development, the pericardial cavity forms above the yolk sac, and the heart occupies the entire pericardial cavity (Capriello et al., 2020). The most common cardiac abnormality in larvae exposed to food additives is cardiac edema (Fig. 3). Also, exposure to food additives has been reported to affect heart rate in fish (Wang et al., 2020).

Cardiac edema is a general response to the toxic effect caused by interference with water permeability barriers. The water permeability barrier around the heart maintains osmotic balance against inward diffusion of water. Edema can occur due to impairment of renal excretion accompanied by decreased blood flow (Jiang et al., 2020). Also, cardiac edema has been reported in some studies to be the result of severe heart rate reduction. Cardiac edema is also thought to be a consequence of immobility or paralysis, and decreased heart rate is a side effect of edema (Lefebvre et al., 2004).

Several tests are reported in the literature that is used to establish cardiotoxicity. Cardiotoxicity of fish larvae can be evaluated by measuring the heart rate, its depression interval and recording the dynamics of heart movement under a microscope. Also, sequential images of electrocardiograms that have been video recorded can be used to measure the variance of cardiac cycles and are used to characterize cardiac motion Kymograph (Bailone et al. 2022).

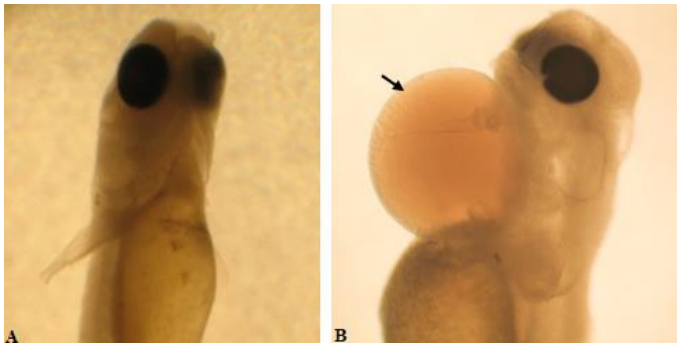

Fig 3. *Hypophthalmichthys molitrix* larvae 72 post fertilization. **A**: normally developed larvae. **B**: larvae with cardiac edema (arrow). Ristea and Zarnescu; unpublished observations.

Food additives may induce cardiac repolarization causing inhibition of heart rate in patients with cardiac risk factors. Thus, *in vivo* toxicity testing of food additives on fish becomes very useful (Wang et al., 2020).

Microscopic anomalies

Generally, anomalies that are evident macroscopically are also confirmed microscopically after histological analysis. In order to visualize the abnormalities caused by food additives, it is necessary to process the larvae for embedding in paraffin according to standard histological protocols for light microscopy. For instance, Capriello et al. (2020) observed skeletal muscle lesions in larvae intoxicated with E150d. It was observed that the lesions are dose dependent because at the concentration of 0.3 g/L the muscle fibers were slightly disorganized, and at the concentration of 0.6 g/L the tissue is much more affected, the muscle fibers are sparse and separated by large spaces. The changes that occur at the level of muscle fibers can also be correlated with the decrease in swimming performance. Also, in larvae treated with E150d, histological analyzes revealed the presence of pericardial edema, the pericardium being enlarged and approximately twice as large as that of normal larvae. In addition, the heart cavity appears swollen and is surrounded by colorless fluid (Capriello et al., 2020).

Kiziltan et al. (2022) observed that larvae exposed to carmoisine dye exhibit degeneration and necrosis of neurons. These effects increase with increasing concentration of carmoisine.

Table 1 Effects reported in the literature for food additives tested on fish

Trial/specias	Tested substances	Exposure duration	Exposure dose	Toxicity	Reference
Danio rerio	Allura red (E129)	72 h.p.f.	0.5, 10, 20, 30, 50, 100 mM	Teratogenic effects (cardiac edema, yolk sac edema, spinal anomalies), mortality	Jiang et al., 2020
		4 d.p.f.	0.05, 0.1, 0.5, 1, 2, 5, 10 g/L	Developmental toxicity, teratogenic effects (cardiac edema, darkened yolk sac, body curvature)	Duy-Thanh et al., 2023
	Amaranth	72 h.p.f.	0.5, 10, 20, 30, 50, 100 mM	Teratogenic effects (cardiac edema, yolk sac edema, spinal anomalies), mortality	Jiang et al., 2020
	Azorubine (E122)	4 d.p.f.	0.1, 0,5, 1, 2, 5, 10, 20 g/L	Reduced larval locomotion	Duy-Thanh et al., 2023
	BDE-47	96 h.p.f	1, 5, 10, 50 and 100 μM	Teratogenic effects (cardiac edema, head deformity, short tail, bent spine, lower jaw deformity, yolk sac deformity)	Parsons et al., 2019
	Brillant blue FCF (E133)	4 d.p.f.	0.1, 0.5, 1, 2, 5, 10, 20, 30, 50 g/L	Lower survival rate, teratogenic effects (ruptured yolk sac)	Duy-Thanh et al., 2023
		9 d.p.f.	0.2%, 0.02%	Increased heart rate	Wang et al., 2020
	Butyl paraben	120 h.p.f.	1, 2, 5 μM	Inhibited swimming behaviors, decreased acetylcholinesterase activity, disruption of the hypothalamic-pituitary interrenal axis	Liang et al., 2023
	Butylated hydroxyanisole, butylated hydroxytoluene	5 d.p.f.	1, 2, 5, 10, 20, 50, 100, 200 μM, 2 mL	Teratogenic effects (uninflated swim bladder, cardiac edema, spinal curvature, yolk deformations)	Yang et al., 2018
	Calcium chloride	96 h.p.f.	1,000; 2,000; 4,000; 8,000; 16,000 ug/ml	Mortality, teratogenic effects (yolk sac anomalies, tail anomalies), cardio toxicity, neurotoxicity	Bailone et al., 2022
	Caramel dye (E150d)	72 h.p.f.	0.3, 0.6 g/L	Teratogenic effects (cardiac edema, tail anomalies), mortality	Capriello et al., 2020
	Carmoisine	6 d.p.f.	4, 50, 100, 200, 400, 800, 1200, 1600, 2000 ppm	Teratogenic effects (cardiac edema, yolk sac edema, tail anomalies)	Kiziltan et al., 2022
	Cinnamaldehyde	96 h.pf.	0.017, 0.035, 0.07, 0.14, 0.28, 0.56 ug/ml	Teratogenic effects (yolk sac edem, body malformation, cardiac edema), mortality, developmental delay	Alves et al., 2021
	Cochineal Red E120	9 d.p.f.	0.2%, 0.02%	Increased heart rate	Wang et al., 2020
		72 h.p.f.	1.2 g L−1	Reduced oxidative stress susceptibility	Napolitano et al., 2022
	Curcumin	120 h	5, 7.5, 10, 12.5 and 15 μM	Teratogenic effects (cardiac edema, spinal anomalies, yolk sac anomalies)	Wu et al., 2007
	Erythrosine	10 d.p.f.	0.001, 0.005, 0.01, 0.02, 0.04, 0.05, 0.1 %	Inhibited swim bladder, teratogenic effects (yolk sac edema, curved body axis)	Gupta et al., 2019

	Erythrostominone	96 h.p.f.	1.875, 3.75, 7.5, 15, 30, 50 mg L^{-1}	Teratogenic effects (yolk sac edema, cardiac edema), affecting swimming behavior, mortality	Abe et al., 2017
	Ethyl paraben	120 h.p.f.	20, 50, 100 µM	Inhibited swimming behaviors, decreased acetylcholinesterase activity, disruption of the hypothalamic-pituitary interrenal axis	Liang et al., 2023
	Methyl paraben	120 h.p.f.	20,100, 200 µM	Inhibited swimming behaviors, decreased acetylcholinesterase activity, disruption of the hypothalamic-pituitary interrenal axis	Liang et al., 2023
		96 h.p.f.	1, 10, 30, 60, 80 mg/L	Teratogenic effects (notochord curvature, cardiac edema, yolk sac edema), behavioral changes	Merola et al., 2020
	Monosodium glutamate (E621)	4 d.p.f.	5, 10, 15, 20, 35, 50 g/L	Teratogenic effects (cardiac edema, darkened yolk sac)	Duy-Thanh et al., 2023
	Patent blue E131	72 h.p.f.	1.2 g/L	Teratogenic effects (tail anomalies, cardiac edema, head anomalies), increased heart rate	Motta et al., 2019
	Ponceau red (E124)	72 h.p.f.	1.2 g/L	Teratogenic effects (tail anomalies, cardiac edema, head anomalies), increased heart rate	Motta et al., 2019
		72 h.p.f.	1.2 g L−1	Reduced oxidative stress susceptibility	Napolitano et al., 2022
	Propyl paraben	120 h.p.f.	2, 5, 10 µM	Inhibited swimming behaviors, decreased acetylcholinesterase activity, disruption of the hypothalamic-pituitary interrenal axis	Liang et al., 2023
	Quinoline yellow (E104)	4 d.p.f.	0.005, 0.02, 0.1, 0.5, 2, 10, 20, 50 g/L	Teratogenic effects (pericardial edema, darkened yolk sac, body curvature), mortality	Duy-Thanh et al., 2023
	Sodium benzoate (E211)	4.d.p.f.	10, 25, 50, 100, 250 mg/L	Decreased swimming activity	Bichara et al., 2013
		24 h	10, 100, 500, 1000, 2000 ppm	Teratogenic effects (gut anomalies, pronephros anomalies, cardiac edema)	Tsay et al., 2007
		4 d.p.f.	0.002, 0.005, 0.01, 0.02, 0.05, 0.1 g/L	Teratogenic effects (darkened yolk, cardiac edema)	Duy-Thanh et al., 2023
	Sunset yellow	7 d.p.f.	0.1, 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 100 mM	Teratogenic effects (cardiac edema, yolk sac edema), developmental toxicity	Joshi and Pancharatna, 2018
		72 h.p.f.	5, 10, 20, 30, 50, 100 mM	Teratogenic effects (cardiac edema, yolk sac edema, spinal defects), mortality	Jiang et al., 2020
		4 d.p.f.	0.1, 0.5, 1, 2, 5, 10, 20 g/L	Developmental toxicity, teratogenic effects (cardiac edema, darkened yolk sac, body curvature)	Duy-Thanh et al., 2023
	Tartrazine yellow (E102)	10 d.p.f.	0.1, 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 75, 100 mM	Teratogenic effects (tail bending, cardiac and yolk sac edema), mortality	Joshi and Pancharatna, 2017
		72 h.p.f.	5, 10, 20,	Teratogenic effects (cardiac	Jiang et al.,

			30, 50 mM	edema, yolk sac edema, spinal defects), mortality	2020
		10 d.p.f.	0.01, 0.05, 0.1, 0.5%	Inhibited swim bladder development, teratogenic effects (axial and dorsal curvature, bent tail, cardiac edema, mouth deformities)	Gupta et al., 2019
		96 h.p.f.	50, 500 mg/L	Teratogenic effects (head malformations, tail sprain, cardiac edema, yolk edema)	Silva and Fracacio, 2023
		72 h.p.f.	1.2 g/L	Teratogenic effects (tail deviation, cardiac edema, head anomalies)	Motta et al., 2019
	TBBPA	96 h.p.f	0.18, 0.46, 0.92, 1.38 and 2.7 µM	Mortality, teratogenic effects (yolk sac edema, head anomalies,spinal anomalies,smaller eyes)	Parsons et al., 2019
	Tert-butyl hydroquinone	5 d.p.f.	1, 2, 5, 10, 20, 50, 100, 200 µM, 2 mL	Teratogenic effects (uninflated swim bladder, cardiac edema, spinal curvature, yolk sac anomalies)	Yang et al., 2018
	Zinc oxide nanoparticles	96 h.p.f.	0.01, 0.1, 1, 10 mg/L	Teratogenic effects (cardiac edema, tail edema, yolk sac edema)	Choi et al., 2016
Mugilogobius chulae	Zinc oxide nanoparticles	6 days	1, 10, 25, 50 mg/ml	Mortality, teratogenic effects (spinal bending, cardiac edema, tail edema, S-shaped body, etc.)	Li et al., 2018
Oplegnathus punctatus	sucrose	30, 60, 90, 120, 150, 180 min.	0.5, 1, 1.5, 2, 2.5, and 3 M	Incomplete tail development (short and thick tail), mortality	Li et al., 2023

BDE-47: 2,2',4,4'-Tetrabromodiphenyl ether; TBBPA: Tetrabromobisphenol A

Mortality and LC_{50}

Determination of mortality percentage is an essential parameter that highlights the toxic effect of food additives on fish larvae. Increased mortality can be associated with the high percentage of morphological anomalies. Many experiments that aimed to test the toxicity on fish larvae revealed a significant increase in mortality caused by different food additives, such as allura red, amaranth (Jiang et al., 2020), brilliant blue FCF (Duy-Thanh et al., 2023), caramel dye (Capriello et al., 2020), erythrostominone (Abe et al., 2017), quinoline yellow (Duy-Thanh et al., 2023), zinc oxide nanoparticles (Choi et al., 2016) etc. (Table 1).

Another parameter that tests the toxicity of a chemical compound is the median lethal concentration (LC_{50}) (Ghosh and Saha, 2022). LC_{50} is the amount or concentration of a chemical compound that can cause death in 50% of organisms (Adamson, 2016).

The LC_{50} is influenced by various factors, such as the method used for acute toxicity testing, purity percentage of the tested substance, and the age and size of the organisms used in toxicity tests. Also, environmental factors such as temperature, pH, alkalinity and turbidity can influence the toxicity of a compound. Organisms can be exposed during experiments to several stress factors at the same time that can interact with each other and cause different synergistic effects. Therefore, these aspects explain the differences between LC_{50s} reported for the same substance in different articles (Islam et al., 2021). Table 2 contains LC_{50s} reported in the literature for food additives tested on fish.

Determination of body length

The body length is an essential parameter that highlights the growth of fish larvae (Yang et al., 2018). Generally, measurements of larvae are made individually using a camera attached to a stereomicroscope.

Body length is usually estimated as notochord length (measurement from the tip of snout to end of notochord) at the preflexion stage, but also as standard length (measurement from the tip of the snout to the posterior end of the hippural plate) afterwards (Rasmussen et al., 2022).

It has been reported in the literature that the size of intoxicated fish larvae is influenced by the tested substance. For instance, following the exposure of *Danio rerio* embryos to sunset yellow dye, a correlation was reported between the dye concentration used and the size of the larvae, their size being inversely proportional to the dye concentration (Joshi and Pancharatna, 2018). Thus, the change in the size of the larvae exposed to different substances and chemical compounds highlight their toxic effect.

Swimming behavior

The evaluation of the swimming mode can explain the toxic potential of different substances on the neurodevelopment of fish larvae. The parameters used for the evaluation are total movement distance and average speed, spontaneous movement (alternating tail bending or coiling), touch response, free swimming activity, swimming in response to alternating light-to-dark (Wang et al., 2013). The appearance of malformations also affects the locomotor activity of the larvae, so changes in swimming behavior can be correlated with the body malformations.

The swim bladder is an important organ in fish that plays a role in maintaining body density and buoyancy (Yang et al., 2018). Abe et al. (2017) analyzed swim bladder inflation in tested larvae and observed that approximately 10% of tested larvae failed to inflate them. Thus, it is possible that the malformation of the yolk sac is associated with the hypoactivity of the larvae (Abe et al., 2017). Some authors associate the locomotor impairment of the larvae with metabolic costs involved on detoxification processes (Andrade et al., 2016). However, many authors associate locomotor disorders with neurological lesions produced by the tested substances (Abe et al., 2017).

However, in many studies it is reported that quantifying the swimming activity of fish is useful for assessing the toxicity of compounds (Bichara et al., 2013; Abe et al., 2017; Capriello et al., 2020; Li et al., 2023; Liang et al., 2023).

Table 2 LC_{50} reported in the literature for food additives tested on fish

Tested substances	Chemical formula	LC_{50}	References
Allura red - E129	$C_{18}H_{14}N_2Na_2O_8S_2$	47.42 mM	Jiang et al., 2020
		1.84 x 10^3 mg/L	Duy-Thanh et al., 2023
Amaranth - E123	$C_{20}H_{11}N_2Na_3O_{10}S_3$	39.86 mM	Jiang et al., 2020
Azorubine - E122	$C_{20}H_{12}N_2Na_2O_7S_2$	3.97 x 10^3 mg/L	Duy-Thanh et al., 2023
BDE-47	$C_{12}H_5Br_5O$	-	Parsons et al., 2019
Brilliant blue FCF -E133	$C_{37}H_{34}N_2Na_2O_9S_3$	-	Duy-Thanh et al., 2023
Butyl paraben	$C_{11}H_{14}O_3$	-	Liang et al., 2023
Butylated hydroxyanisole	$C_{11}H_{16}O_2$	99.7 µM	Yang et al., 2018
Butylated hydroxytoluene	$C_{15}H_{24}O$	>200 µM	Yang et al., 2018
Calcium chloride	$CaCl_2$	0.35%	Bailone et al., 2022
Carmoisine - E122	$C_{20}H_{12}N_2Na_2O_7S_2$	1230.53 ppm	Kiziltan et al., 2022
Cinnamaldehyde	C_9H_8O	0.311 µg/mL	Alves et al., 2021
Curcumin	$C_{21}H_{20}O_6$	5 µM	Wu et al., 2007
Erythrostominone	$C_{17}H_{16}O_8$	26.68 mg/L	Abe et al., 2017
Methylparaben	$C_8H_8O_3$	72.67 mg/L	Merola et al., 2020
Monosodium glutamate - E621	$C_5H_8NO_4Na$	19.2–21.1 × 103	Duy-Thanh et al., 2023
Quinoline yellow - E104	$C_{18}H_{11}NO_2$	6.89 × 103	Duy-Thanh et al., 2023
Sodium benzoate -E211	$C_{14}H_{10}Na_2O_4$	461 mg/L	Bichara et al., 2013
		1400-1500 ppm	Tsay et al., 2007
		26.9 mg/L	Duy-Thanh et al., 2023
Sunset yellow - E110	$C_{16}H_{10}N_2Na_2O_7S_2$	42.57 mM	Joshi and Pancharatna, 2018
		0.224 mg/L	Jiang et al., 2020
		5.27 x 10^3	Duy-Thanh et al., 2023
Tartrazine - E102	$C_{16}H_9N_4Na_3O_9S_2$	29.4 mM	Joshi and Pancharatna, 2017
		47.10 mM	Jiang et al., 2020
TBBPA	$C_{15}H_{12}Br_4O_2$	0.9 µM	Parsons et al., 2019
Tert-butyl hydroquinone - E319	$C_{10}H_{14}O_2$	55.4 µM	Yang et al., 2018
Nano zinc oxide	Nano-ZnO	45.40 mg/L	Li et al., 2018

Conclusions

Food additives are important and essential tools for food industry, thus more toxicity tests are needed. Fish larvae are ideal organisms for testing food additives because they are permeable to small molecules during organogenesis and the studies based on the classification of teratogenic and non-teratogenic substances have demonstrated a high correlation of toxic response between fish and mammals. Therefore, fish larvae are relevant, reliable, controllable and reproducible models for studying the effects of chemical substances on living organisms, including humans.

Despite the improvements in legislation and the production of food additives, there are still many controversies regarding their use in the food industry. The fact that most of the food additives tested on fish larvae induced morphological changes, such as spinal curvature, yolk sac edema, cardiac edema, but also a significant mortality at some concentrations, highlights

that the use of certain food additives must be additionally tested and reconsidered.

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