Zebrafish as an animal model for food safety research: trends in the animal research

by Bailone, R.L., Aguiar, L.K.D., Roca, R.D.O., Borra, R.C., Corrêa, T., Janke, H. and Fukushima, H.C.S.

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"Zebrafish as an Animal Model for Food Safety research: trends in the animal research"

ABSTRACT

Toxicity studies in mammals continue to be the most appropriate model for predicting risk in humans, but they tend to be expensive and time-consuming. In the aftermath of the genetic sequencing of zebrafish (*Danio rerio*), that species showed to be highly genetically homologous to humans. The use of the zebrafish model to assess food toxicity is already a reality as it is capable of biological processes difficult to reproduce *in vitro.* Studies of complex mechanisms of absorption, distribution, metabolism and excretion as well as cellular and tissue interactions are of great information value resulting in time, space and cost savings, when compared to studies with rodents. This review addresses the relevance of zebrafish model in food safety research, both in the use of ingredients and innocuous food additives as well as for establishing levels of safe food contaminant residues present in the environment. Toxicological screening using the zebrafish model integrate the evaluation of teratogenicity, cardiotoxicity, hepatotoxicity, genotoxicity, neurotoxicity, endocrinetoxicity, reproductive and behavioral aspects. These are important endpoints for food safety assessment, which take substantially less time than in mammalian tests. Furthermore, it serves well as a screening test follow-up for validating favorable results in murine models, hence accelerating the risk assessment process of products submitted for approval and registration, prioritizing safe compounds and reducing unnecessary costs in subsequent mammalian studies. In conclusion, zebrafish model can be a useful tool for food safety tests, however, additional studies are needed to further validate this model for registration of new food ingredients and additives.

Key-words: Additives; Contaminants; 3R; Food Biotechnology; Toxicology.

Introduction

Currently, toxicity affecting humans, animals and the environment are the main causes of conflict during the process of approval of food ingredients and additives. In order to modernize and accelerate the registration process for new molecules and enable safer and more nutritious products to reach the market faster, zebrafish model has become an unprecedented tool. Zebrafish (*Danio rerio*) is considered an excellent animal model for investigating the effects of food additives on human and animal health, and for quickly and economically assessing the safety of new compounds. It also works well for the detection of safe levels of contaminants present in the environment which might leave residues in food.

The interest in the study of food toxicology is evident because humankind depends on nutrition by virtue of heterotrophic metabolism. Fast advances in the sciences of biochemistry, molecular biology, cell culture techniques, computer and bioinformatics has enabled the identification and characterization of potential toxicants in food (Knudsen et al. 2015; Ahuja and Sharma 2014; Sun et al.2012; Houck and Kavlock 2008; Barlow et al. 2002; Eisenbrand et al. 2002; Paustenbach 2000).

Rodent animal models have been used for testing food toxicity; however, in recent years the use of rodents in animal testing has presented some limitations regarding high costs, low throughput readouts, inconsistent responses, ethical issues and concerns of extrapolability to humans. Alternatively, lower hierarchy surrogate animal models (e.g. *Drosophila melanogaster*; *Caenorhabditis elegans* or *Danio rerio*) have been used in an effort to integrate organotypic systems and stem cell-based experiments. The value of using alternative sub-mammalian vertebrate and invertebrate models became evident by the surprising discovery of the high degree of gene homology between humans and zebrafish, fruit flies or nematodes (Raldua and Pina 2014; Chakravarthy et al. 2014; Prussing, Voigt and Schulz 2013; Sun et al.

2012; Pandey and Nichols 2011; Leung et al. 2008; Bier 2005; Barbazuk et al. 2000). The alternative models offer an advantage in terms of ethical concerns, high throughput and genetic manipulation over traditional rodent models (Ahuja and Sharma 2014; Sun et al. 2012; Pandey and Nichols 2011; Giacomotto and Ségalat 2010; Houck and Kavlock 2008). Whereas tests with mammals could become a confirmatory model of the findings, substantially reducing their use in research. Despite the advantages, there are still numerous challenges in various disciplines of food toxicology (Gosslau 2016).

In addition to the relevance of the zebrafish model applied to toxicological research, the rapid development of new substances through bioengineering techniques, requires that fast and efficient methods for screening new compounds are developed and standardized. The zebrafish model allows for tests to be carried out since the early stages of the embryonic development until reaching the adult stage in only three months. Furthermore, the daily expenditure with the *Danio rerio* cost US$ 0.16 when compared toUS$2.15 in rats (Santos 2018). The use of zebrafish model also contributes for validating those more favorable outcomes resulting from murine models. This is because murine modules tend to be costly, space and time consuming. Thus, the zebrafish model can speed up the risk assessment process for food products, particularly novel foods, which are subject to lengthy approval and registration, hence prioritizing safe compounds and reducing unnecessary costs in subsequent mammalian studies.

The relevance of the zebrafish model and its widespread application worldwide is now housed under a repository of information such as Zebrafish Information Network (ZFIN). Data on zebrafish parameters such as mortality, growth retardation, craniofacial malformations, yolk sac edema, pericardial edema, meningeal edema, peripheral ischemia and disruption of erythropoiesis, arrested gill development, impaired swim bladder inflation, altered apoptosis, altered apoptosis, decreased number of neurons in the brain, inhibition of fin regeneration, inhibition of common cardinal vein regression, reduced heart size and ventricular standstill, can

be easily accessed.

The purpose of this article is to present a most updated review of the literature on the use of zebrafish model. It highlights its applications for the food manufacturing industry particularly in food safety analysis. It aims to bring to light the research findings that help in establishing the levels of safe contaminant residues in food ingredients and additives.

Application in Food Safety Research

The widely use of zebrafish as an *in vivo* vertebrate organism model in a variety of research fields, such as drug discovery and toxicology, results from the striking similar toxicity profile between humans and the fish. Zebrafish sequenced genetic information has high degree of homology (over 70%) due to its substantial physiological, anatomic, and genetic homology (Raldua and Pina 2014; Hill et al. 2005; Howitz et al. 2003; Barbazuk et al. 2000) to that of human genes (Howe et al. 2013) (Table 1).

TABLE 1

Among animal model research systems that are receptive to the screening of toxic substances, the zebrafish model stands out for their highly conserved integrative physiology (McRae and Peterson 2015). The hematopoietic system of zebrafish is highly similar to that of humans and consists of the same cell types (erythrocytes, neutrophils, eosinophils, lymphocytes, macrophages and so on) (Jagannathan-Bogdan and Zon 2013). Cardiovascular physiology is also highly conserved between humans and zebrafish at anatomical, cellular, and membrane biology levels. Many human cardiovascular drugs have been shown to have identical effects on zebrafish physiology and numerous human cardiovascular disorders have been reiterated in zebrafish genetic models (Asnani and Peterson 2014). Similarly, close correlations have been observed for hepatotoxicity, nephrotoxicity, and reproductive toxicity, in which all toxins known in preclinical mammalian or human models presented similar effects in zebrafish

(Driessen et al. 2004; Ducharme et al. 2014).

These similarities have led researchers to use zebrafish as an alternative to mammals in different studies that rely on biological tests for the elucidation of events, such as modulation of diseases, drug screening, target identification, pharmacology, toxicology, physiology, behavior, among others. It is also amenable to gene manipulation, is cost low, has a short progeny time, and is particular well suited for high-throughput screening as well as transcriptonic and proteomic studies (Chakravarthy et al. 2014; McGrath and Li 2008; Love et al. 2004). Thus, the use of zebrafish, as a model of testing that evaluate a likely toxicity of food compounds is of extreme relevance.

a) Toxicity

Numerous studies confirm that zebrafish and mammalian toxicity profiles are surprisingly similar, with a different kind of substances, as geladanamycin antibiotic, ethanol (ethyl alcohol), dexamethasone (medicinal product belonging to the class of corticosteroids), acetaminophen (analgesic and antipyretic drug properties), didemnin B, doxorubicin (drug widely used in cancer chemotherapy), aspirin (acetylsalicylic acid), amiodarone (drug of the broad spectrum class III antiarrhythmics group and a potent vasodilator), tacrine (drug used by medicine as a reversible acetylcholinesterase inhibitor), 4-ipomeanol (lung pre-toxin isolated from sweet potato infected with the fungus *Fusarium solani*), caffeine, cyclosporine A (immunosuppressant drug of the calcineurin inhibitor class isolated from the fungus *Tolypocladium inflatum)*, amongst others (Driessen et al. 2015; Boyd et al. 2015; Makhija and Jagtap 2014; Zhang, Willett and Fremgen 2003 *apud* Kari, Rodeck and Dicker 2007).

For being more practical, efficient and cheaper than the rodent model, the zebrafish one is able to accelerate and reduce the cost of the research process, allowing experiments to be carried out in a matter of months, using fewer resources, rather than years as it is the case of

other mammal species (Zorzetto and Guimarães 2013). Thus, the use of zebrafish has been widely disseminated in international research, and is currently considered an unprecedented tool as an animal model.

Zebrafish's peculiar biology also allows the researcher to readily access all stages of its development. Since zebrafish larvae are transparent, they are ideal for studies on organ morphology by *in vivo* imaging techniques in addition to more detailed studies by immunohistochemistry or in situ hybridization in real time (Raldua and Pina 2014; McGrath and Li 2008; Howitz et al. 2003).

The embryos' and larvae's transparency enable the visualization in real time of development of diseases. For a researcher, the ability to examine the beginning and the course of a pathological process *in vivo* is a remarkable feature of zebrafish models (Figure 1).

FIGURE 1

Hill et al. (2005) postulated that the zebrafish model is expected to help with the evaluation and determination of the toxicological analysis. Zebrafish's translucent body and short lifecycle allows direct *in vivo* evaluation of the toxicity of molecules. Also due to its transparency, there is currently available software and methods capable of measuring parameters such as changes in physiology: liver toxicity (Figure 2), intestinal tract velocity, heart rate and blood flow (Figure 3); and behavioral changes (speed, number of movements, downtime, compulsive behavior) of each animal evaluated, refining the quality of research results.

FIGURE 2

FIGURE 3

This teleost present great sensitivity to chemical products since they can quickly absorb the compounds which are diluted in the water. The fish can also accumulate residues in different tissues, mainly in the Central Nervous System (Sant'Anna et al. 2011). Furthermore, according

to international ethical protocols, zebrafish larvae studies of up to 120hs after fertilization (h.p.f) are considered to be *in vitro* models which are acceptable as alternative for animal trials (Cornet et al. 2017). As a result, several toxicity tests have already been described (Lawrence et al. 2016). There are currently OECD-validated standards for zebrafish embryo and larval use (OECDILIBRARY 2019; OECD 210 2013; OECD 236 2013; OECD 229 2012; OECD 420 2001). The OECD 236 toxicity test method, per example, determines the acute lethal effects of chemicals (including industrial chemicals, pharmaceuticals, biocides, pesticides, biocides, inorganic chemicals, etc.) in the pre-larval stages of zebrafish. Zebrafish's newly fertilized eggs are exposed for 96 hours to chemicals; lethal effects are recorded and used to calculate the lethal concentration of 50% of embryos (OECD 236 2013).

b) Food Ingredients, Additives and Preservatives

In recent years, many studies have been conducted with the zebrafish model testing the toxicity of food ingredients, additives and preservatives such as sodium nitrite and nitrate (Bailone et al. 2018; Fukushima et al. 2018, Keshari et al. 2016; Simmons et al. 2012), methylparaben (Dambal et al. 2017) and sodium benzoate (Tsay et al. 2007).

Keshari et al. (2016) showed that increasing the time of exposure to nitrite negatively affected survival. Increasing the concentration of nitrite also adversely affected the survival rate, whereas nitrate did not. For embryos that survived nitrite exposure, various defects could occur, including pericardial and yolk sac edema, swim bladder non-inflation, and craniofacial malformation. Their results indicated that the zebrafish model was a convenient system for studying the teratogenic potential of nitrite. Dambal et al. (2017), studied vitellogenin in embryo-larval stages of zebrafish exposed to methylparaben and found $LC_{50\%}$ in 428 µM (0.065 mg/L). An increase on the exposure concentration resulted in decreased in both heart and hatching rates. The exposure to sub-lethal concentration (100 Mm) increased the Vtg-I gene

175 expression.

176 Tsay et al. (2007) studied the effects of sodium benzoate in zebrafish larvae. They
177 showed that treatment with sodium benzoate led to misalignment of muscle fibers, motor
178 neuron innervations, excess acetyl-choline receptor cluster and defective pronephric tubes.
179 Based on their observations, they suggested that sodium benzoate is able to induce neurotoxicity
180 and nephrotoxicity of zebrafish larvae.

181 Other food additives have also been tested using zebrafish model, such as food colorants
182 Sunset Yellow (E110) and tartrazine. According to Joshi and Pancharatna 2018; Joshi and Katty
183 2018, the developmental toxicity/teratogenic potential of Sunset Yellow (E110) on zebrafish
184 embryos was confirmed and caution was issued on its consumption/usage/frequency
185 particularly by gestating women, as the level and combination of food additives in food and the
186 pattern of food consumption was a variable among individuals. In relation exposure of embryos
187 to tartrazine, concentration of < 10 mM showed to have no effects. Yet, those of 20 to 30 mM
188 caused tail bending, cardiac and yolk sac edema in 50% of the larvae. At 30 to 50 mM embryos
189 had a lower heart rates and presented the afore mentioned deformities, but with mortality within
190 96 to 144 hpf; development ceased completely at 75 to 100 mM concentration. The NOEC and
191 $LC_{50\%}$ were recorded at 5 and 29.4 mM dose, respectively.

192 Neurotoxicity has also been tested on flavor enhancers, such as monosodium glutamate,
193 concluding that exposure at early embryonic stage increased brain cell damage and risk of
194 behavior changes (Kurnianingsih et al. 2016). Sweeteners such as aspartame and saccharin have
195 been tested using the zebrafish model. As the aspartame concentrations increased, different
196 observable deformities were formed in the embryo. Regarding saccharin-fed zebrafish they
197 showed an increase in the atherogenic serum lipid profile (Weerasooriyagedara 2018; Kim, Seo
198 and Cho 2011).

199

c) Agrochemicals

When good agricultural practices are not followed closely, agrochemical residues can be found in foods. That leads to serious public health problems which can be both through environmental exposure in the water or bioaccumulation in animals. Many agrochemical products have already been tested with the zebrafish model, such as glyphosate (Bridi et al. 2017; Sulukan et al. 2017; Roy, Carneiro and Ochs 2016; Roy et al. 2016), parathion (Roex, Keijzers and Van Gestel 2003), endosulfan (Velasco-Santamaría, Handy and Sloman 2011; Willey and Krone 2001), diphenoconazole (Mu et al. 2013), dithiocarbamate (Haendel et al. 2004), carbaryl (Todd and Van Leeuwen 2002), toxaphene (Ree and Payne 1997), chlorpirifos (Levin et al. 2003), matathion (Cook, Paradise and Lom 2005), atrazine (Blahová et al. 2013; Wiegand et al. 2001) and pyrethroids, (Ansari and Ahmad 2010).

Bridi et al. (2017), studying the effects of Glyphosate and Roundup®, showed that those substances could alter both morphological and behavioral parameters in zebrafish, hence suggesting common mechanisms of toxicity and cellular response. In zebrafish larvae those substances altered locomotion and aversive behavior as well as decreased ocular distance. Yet in zebrafish adults, locomotion was also reduced and impairment in memory and reduced aggressive behavior was observed. Sulukan et al. (2017) showed that glyphosate exposure caused an inhibition effect of carbonic anhydrase enzyme whose decreased activity lead to an increase in CO_2 and respiratory acidosis in the whole body resulting in producing of Reactive Oxygen Species (ROS) in the gills. The increased and elevated presence of ROS was attributed to cause malformations due to the cellular apoptosis. Furthermore, Roy et al. (2016) observed neurotoxicity when zebrafish was in contact with glyphosate causing a serious of disruptions such as loss of delineated brain ventricles and cephalic regions in embryos, decreased gene expression in the eye as well as fore and midbrain regions. Roy et al. (2016) also showed that glyphosate caused changes to the atria and ventricle and decreased heart rate, altered the

Commented [LDA1]: Et al. Here?

vasculature in the body, and expression of Mef2 in early myocardial precursors. They concluded that glyphosate and the Roundup® formulations were developmentally toxic to the forebrain and midbrain, and that glyphosate affected the cardiovascular system thus being both developmentally toxic to the zebrafish heart.

Blahová et al. (2013), studied one of the most used pesticides worldwide, the herbicide atrazine, which is frequently detected in surface water. They found that the changes in antioxidant enzyme activities could be an adaptive response to protect the fish from the atrazine-induced toxicity. Moreover, Difenoconazole, a widely used fungicide was investigated by Mu et al. (2013) who demonstrated that it induced many abnormalities during the zebrafish embryonic and larvae stages and caused growth inhibition of adult zebrafish after 14 days of exposure. Velasko-Santamaría, Handy and Sloman (2011) analyzed how the insecticide endosulfan affected the health variables in zebrafish. They proposed that sublethal exposure to endosulfan caused adverse sublethal effects in adult *D. rerio,* and effected the development of their offspring. Furthermore, Ansari and Ahmad (2010), studied the toxicity of pyrethroid Lambda-cyhalothrin and Neemgold to the embryo of zebrafish. They found that toxicity was time as well as concentration-dependent. Embryos were more sensitive to Lambda-cyhalothrin than to Neemgold.

Cook, Paradise and Lom (2005), proved that the pesticide malathion reduced survival and growth in developing zebrafish, and concluded that both malathion's action as an acetylcholinesterase inhibitor, and the toxicity of its metabolites might be responsible for malathion's teratogenic effects on fish development. Haendel et al. (2004) researched a biocide widely used in agriculture which is generally applied prior to planting for the control of nematodes, soil pathogens, and weeds. They concluded that developing zebrafish were sensitive to Sodium Metam (NaM), a dithiocarbamate. Roex, Keijzers and Van Gestel (2003), exposed zebrafish to sublethal concentrations of the organophosphorus pesticide parathion for 250 days

Commented [LDA2]: Et al?

Commented [LDA3]: Et al?

250 in a flow-through system. That agrochemical promoted acetylcholinesterase inhibition and
251 increased the food consumption rate.
252
253 ***d) Pharmaceutical residues***
254 In relation to antibiotic residues used in animal husbandry, there is growing concern as
255 those are found in water bodies. As a result of antibiotics such as tetracycline (Zhang, Cheng
256 and Xin 2015), cephalosporin (Zhang et al. 2010), fluoroquinolone (Zhang et al. 2016), Commented [LDA4]: Et al?
257 sulphonamides (Lin, Chen and Chen 2013), amoxicillin and oxytetracycline (Oliveira et al. 2013) Commented [LDA5]: Et al?
258 being administered to animals they end up contaminating food, especially meat products due to
259 residue accumulation.
260 Zhang, Cheng and Xin (2015), analyzed tetracycline toxicity and proved that Commented [LDA6]: Et al?
261 tetracycline could produce oxidative stress and induce apoptosis, which brought about
262 significant developmental delay in zebrafish embryos. Oliveira et al. (2013) investigated the
263 toxicity of amoxicillin and oxytetracycline and demonstrated that oxytetracycline inhibited
264 zebrafish hatching, whereas amoxicillin also caused premature hatching. Lin, Chen and Chen Commented [LDA7]: Et al?
265 (2013), studding the toxicity of sulfonamides, observed that exposure to a low concentration of
266 sulfonamide (0.001mg/L) resulted in characteristic malformations, including pericardial edema,
267 yolk sac edema, hemagglutinations, tail deformation and swim bladder defects.
268
269 ***e) Mycotoxins***
270 The effect of mycotoxins such as zearalenone (ZEA) (Bakos et al. 2013), aflatoxin
271 (Troxel et al. 1997), citrinin and patulin (Wu et al. 2012) have also been conducted.
272 Bakos et al. (2013) referred to the early effects of ZEA exposure being concentration-
273 dependent with $LC_{50\%}$ and $LC_{10\%}$ values of 893 and 335µg/L. In larvae exposed to 500µg/L and
274 above, ZEA induced phenotype changes showing defects in heart and eye development and

upward curvature of the body axis. Regarding a dosage of 250µg/L at 72 h.p.f. the gap in the melanophore streak at the base of the tail fin was missing and the fin fold was abnormal, suggesting disturbance in the development of the adult tail fin primordium. Estrogenic potency was measured on the basis of Vitellogenin (Vtg) protein levels in adults and relative abundance of vitellogenin-1 mRNA (vtg-1) (in both larvae and adult). qRT-PCR in larvae proved to be an appropriate substitute to testes in adult and were sensitive enough to detect ZEA in 0.1µg/L concentrations, which is a value close to levels observed in wastewaters. Developmental defects revealed that besides direct estrogenic effects, ZEA might interact with other ontogenic pathways. Wu et al. (2012) analyzed the nephrotoxicity of citrinin and patulin on zebrafish embryos and found that both mycotoxins caused profound nephrotoxicity in histological structure and biological function of zebrafish embryos as well as the inflammatory pathway and blood rheology might involve in CTN-induced renal impairment.

Another type of toxin analyzed was saxitoxin which is a component of Paralytic Shellfish Poisoning (PSP) and are produced in large quantities during episodes of harmful algae proliferation, a phenomenon known as "red tides" (Lefebvre, Trainer and Scholz 2004). The zebrafish model was used to explore the sublethal effects of a dissolved of saxitoxin on early fish development including sensorimotor function, morphology, and long-term growth and survival. It proved that dissolved algal toxins might therefore have important sublethal effects on vulnerable fish species, as significantly it was evidenced a reduced growth and survival at 18 and 30 days of age.

Commented [LDA8]: Et al?

f) Heavy metals/ semimetals / nonmetals

Chemical contaminants present in the environment and that leave residues in food have been studied, mainly regarding heavy metals such as mercury (Glynn, Norrgren and Müssener

2010; Dave and Xiu 1991), methylmercury (Samson et al. 2001), cadmium (Zhang et al. 2012; Glynn, Norrgren and Müssener 2010; Chan and Cheng 2003; Hen Chow and Cheng 2003; Dave 1985), aluminum (Dave 1985), copper (Pereira, Campos and Bogo 2016, Luzio et al. 2013; Rougier et al. 1996; Dave and Xiu 1991), iron (Dave 1985), nickel and lead (Dave and Xiu 1991), uranium (Labrot et al. 1999), tin (Sisman 2011), zinc (Rougier et al. 1996), and semimetals such as arsenic (Li et al. 2016) as well as transition metals such as cobalt (Dave and Xiu 1991) and nonmetals such as selenium (Zhang et al. 2012).

Samson et al. (2001), studying the effects of methylmercure found that continuous embryonic exposure to 15µg/l caused delayed mortality syndrome (DMS). These larvae hatched normally and appeared normal, but at the onset of Day 3 post-hatch (ph), general activity was severely impaired and by Day 5 ph, larvae were completely moribund. Moreover, many fish had faint heartbeats, presented severely enlarged body cavities and upward flexures of the spinal cord. Most of these larvae were dead by Day 6 ph. Multi- and single-day embryonic exposures to 15µg/l caused reduced swimming activity and prey capture ability, and by Day 4 ph, these larvae also began to show signs of DMS. Samson et al. (2001) study reinforced the idea that functional impairment was a more subtle response to developmental toxicants than mortality or the production of morphological defects.

g) Transgenic foods

Transgenic foods, such as soya and maize, have also been tested using the zebrafish model. Trials evaluating feed intake, growth, stress response and uptake of dietary DNA, also support the feasibility of using zebrafish as a model organism, not only in relation to chemical toxicology, but also to study the safety of whole foods (Sissener et al. 2010). Among the compounds mentioned above, many others have been continuously analyzed in relation to food safety, proving the effectiveness of this in vivo animal research model in the area of food safety

(Bencsik et al. 2018; Mezzomo et al. 2018; Shi et al. 2009; De Oliveira Ribeiro et al. 2008).

Zebrafish is, therefore, the ideal animal model with potential to speed up the regulatory process for approval of food ingredients and additives. Using zebrafish models would also aid in prioritizing cases for compound approval, and reduce unnecessary costs in subsequent mammalian studies. It, thus, allows for a risk assessment of compounds regarding endophytic, teratogenic, cardiotoxic, neurotoxic, hepatotoxic and genotoxic effects. These tests allow for predicting the safety levels and possible impacts of these compounds on human, animal, as well as on the environment. The relevance of the zebrafish use also valuable for (i) the need to modernize the registration process of new agrochemical molecules; (ii) the potential of the zebrafish model in toxicological studies (acute and chronic), and the possibility of biotechnological advances obtained from its use; (iii) current urgency of safer and more advanced products reaching the market faster (iv) economic benefits for toxicological screening with the zebrafish model.

Currently, the research opportunities, such as the use of zebrafish in behavioral neuroscience, are in their infancy when compared to the use of laboratory rodents. As technology advances, mutant zebrafish, morpholinos, high-throughput screening and new bioassays for toxic and therapeutic endpoints in zebrafish will be the norm. For the purpose of toxicology testing, these advances, in addition to the accumulation of genetic and genomic infrastructure, will ultimately provide greater insight into the mechanisms of toxicity of chemicals, as well as aid in the discovery of new drugs for treating human disease.

CONCLUSIONS

Zebrafish is, therefore, the ideal animal model with potential to speed up the regulatory process for approval of food ingredients and additives. Using zebrafish model would also aid in prioritizing cases for compound approval, and reduce unnecessary costs in subsequent

mammalian studies. It, thus, allows for a risk assessment of compounds regarding endophytic, teratogenic, cardiotoxic, neurotoxic, hepatotoxic and genotoxic effects. These tests take into consideration the predicting of the safety levels and possible impacts of these compounds on human, animal, as well as on the environment. The relevance of the zebrafish use is also valuable for:

(i) The need to modernize the registration process of new agrochemical molecules;

(ii) The potential of the zebrafish model in toxicological studies (acute and chronic),

(iii) The possibility of biotechnological advances obtained from its use;

(iv) Filling the gap regarding the current urgency of safer and more advanced products reaching the market faster, and

(v) The economic benefits for toxicological screening with the zebrafish model.

Currently, the research opportunities, such as the use of zebrafish resides in behavioral neuroscience, are in their infancy when compared to the use of laboratory rodents. Yet, as technology advances, mutant zebrafish, morpholinos, high-throughput screening and new bioassays for toxic and therapeutic endpoints in zebrafish would be expected to be norm. For the purpose of toxicology testing, these advances, in addition to the accumulation of genetic and genomic infrastructure, will ultimately provide greater insight into the mechanisms of toxicity of chemicals, as well as aid in the discovery of new drugs for treating human disease.

Zebrafish model can be a useful tool for food safety tests as it saves in research time, space and expenditure. It is an invaluable tool for validating tests carried out using other models. For its practicality, it is also useful as a complementary zebrafish model in food ingredients research. However, more studies are needed to further validate this model for the purpose of registration of new ingredients and food additives.

REFERENCES:

376 Ahuja, V., and S. Sharma. 2014. Drug safety testing paradigm, current progress and future
377 challenges: an overview. *Journal of Applied Toxicology.* 34(6):576-594. doi:10.1002/jat.2935
378
379 Ansari, B. A., and M. K. Ahmad. 2010. Toxicity of pyrethroid Lambda-cyhalothrin and
380 Neemgold to the embryo of zebrafish *Danio rerio* (Cyprinidae). *Journal of Applied*
381 *Bioscience.* 36(1):97-100.
382
383 Asnani, A., and R. T. Peterson. 2014. The zebrafish as a tool to identify novel therapies for
384 human cardiovascular disease. *Disease Models & Mechanisms.* 7:763–767.
385 doi: 10.1242/dmm.016170
386
387 Bailone, R. L., H. S. C. Fukushima, R. O. Roça, T. F. D. Castro, C. Corrêa, T. A. Robeldo,
388 and R. C. Borra. 2018. Acute Toxicity of Healing Salts for Cured Meats. In: *V Simpósio*
389 *Zebrafish como Modelo Animal de Pesquisa*, Universidade Federal do Rio Grande do Norte.
390
391 Bakos, K., R. Kovács, Á. Staszny, D. K. Sipos, B. Urbányi, F. Müller, and B. Kovács. 2013.
392 Developmental toxicity and estrogenic potency of zearalenone in zebrafish (*Danio*
393 *rerio*). *Aquatic Toxicology.* 136:13-21. doi:10.1016/j.aquatox.2013.03.004
394
395 Barbazuk, W. B., I. Korf, C. Kadavi, J. Heyen, S. Tate, E. Wun, and S. L. Johnson. 2000. The
396 syntenic relationship of the zebrafish and human genomes. *Genome Research.* 10(9):1351-
397 1358. doi:10.1101/gr.144700
398
399 Barlow, S. M., J. B. Greig, J. W. Bridges, A. Carere, A. J. M. Carpy, C. L. Galli, and C.
400 Madsen, C. 2002. Hazard identification by methods of animal-based toxicology. *Food and*
401 *Chemical Toxicology.* 40(2-3):145-191. doi:10.1016/S0278-6915(01)00117-X
402
403 Bencsik, D., G. Gazsi, B. Urbányi, B., B. Szende, G. Rácz, A. Véha, and Z. Csenki. 2018.
404 Assessment of subacute genotoxic and histopathological effects of a food flavour ingredient,
405 4-ethylbenzaldehyde (EBA) on zebrafish (*Danio rerio*) model. *Acta Alimentaria.* 47(2):245-
406 251. doi:10.1556/066.2018.47.2.14
407
408 Bier, E. 2005. Drosophila, the golden bug, emerges as a tool for human genetics. *Nature*
409 *Reviews Genetics.* 6(1):9. doi:10.1038/nrg1503
410
411 Blahová, J., L. Plhalová, M. Hostovský, L. Divišová, R. Dobšíková, I. Mikulíková, and Z.
412 Svobodová. 2013. Oxidative stress responses in zebrafish *Danio rerio* after subchronic
413 exposure to atrazine. *Food and Chemical Toxicology.* 61:82-85. doi:10.1016/j.fct.2013.02.041
414
415 Boyd, W., M. V. Smith, C. A. Co, J. R. Pirone, J. R. Rice, K. R. Shockley, J. H. Reedman,
416 and A. Indy. 2015. Developmental effects of the ToxCast™ Phase I and Phase II chemicals in
417 *Caenorhabditis elegans* and corresponding responses in zebrafish, rats, and
418 rabbits. *Environmental Health Perspectives.* 124(5):586-593. doi:10.1289/ehp.1409645
419
420 Bridi, D., S. Altenhofen, J. B. Gonzalez, G. K. Reolon, and C. D. Bonan. 2017. Glyphosate
421 and Roundup® alter morphology and behavior in zebrafish. *Toxicology.* 392:32-39.
422 doi:10.1016/j.tox.2017.10.007
423
424 Chakravarthy, S., S. Sadagopan, A. Nair, and S. K. Sukumaran. 2014 Zebrafish as an *in vivo*
425 high-throughput model for genotoxicity. *Zebrafish.* 11:154–166. doi:10.1089/zeb.2013.0924

Chan, P., and S. Cheng. 2003. Cadmium-induced ectopic apoptosis in zebrafish embryos. *Archives of Toxicology.* 77(2):69-79. doi:10.1007/s00204-002-0411-1

Cook, L. W., C. J. Paradise, and B. Lom. 2005. The pesticide malathion reduces survival and growth in developing zebrafish. *Environmental Toxicology and Chemistry.* 24(7):1745-1750. doi:10.1897/04-331R.1

Cornet, C., S. Calzolari, R. Miñana-Prieto, S. Dyballa, E. Van Doornmalen, H. Rutjes, and J. Terriente. 2017 ZeGlobalTox: an innovative approach to address organ drug toxicity using zebrafish. *International Journal of Molecular Sciences.* 18(4):864. doi:10.3390/ijms18040864

Dambal, V. Y., K. P. Selvan, C. Lite, S. Barathi, and W. Santosh. 2017. Developmental toxicity and induction of vitellogenin in embryo-larval stages of zebrafish (*Danio rerio*) exposed to methyl Paraben. *Ecotoxicology and Environmental Safety.* 141:113-118. doi:10.1016/j.ecoenv.2017.02.048

Dave, G., and R. Xiu. 1991. Toxicity of mercury, copper, nickel, lead, and cobalt to embryos and larvae of zebrafish, *Brachydanio rerio*. *Archives of Environmental Contamination and Toxicology.* 21(1):126-134. doi:10.1007/BF01055567

Dave, G. 1985. The influence of pH on the toxicity of aluminum, cadmium, and iron to eggs and larvae of the zebrafish, *Brachydanio rerio. Ecotoxicology and Environmental Safety.* 10(2):253-267. doi:10.1016/0147-6513(85)90072-7

De Oliveira Ribeiro, C. A., M. D. Nathalie, P. Gonzalez, D. Yannick, B. Jean-Paul, A. Boudou, and J. C. Massabuau. 2008. Effects of dietary methylmercury on zebrafish skeletal muscle fibres. *Environmental Toxicology and Pharmacology.* 25(3):304-309. doi:10.1016/j.etap.2007.10.033

Driessen, M., A. P. Vitins, J. L. Pennings, A. S. Kienhuis, B. Van De Water, and L. T. Van Der Ven. 2015. A transcriptomics-based hepatotoxicity comparison between the zebrafish embryo and established human and rodent in vitro and in vivo models using cyclosporine A, amiodarone and acetaminophen. *Toxicology Letters.* 232(2):403-412. doi:10.1016/j.toxlet.2014.11.020

Ducharme, N. A., D. M. Reif, J. A. Gustafsson, and M. Bondesson. 2014. Comparison of toxicity values across zebrafish early life stages and mammalian studies: implications for chemical testing. *Reprodutive Toxicology.* 55:3–10.

Eisenbrand, G., B. Pool-Zobel, V. Baker, M. Balls, B. J. Blaauboer, A. Boobis, and J. Kleiner. 2002. Methods of *in vitro* toxicology. *Food and Chemical Toxicology.* 40(2-3):193-236. doi:10.1016/S0278-6915(01)00118-1

Fukushima, H. C. S., R. L. Bailone, T. F. D. Castro, T. Corrêa, T., T. A. Robeldo, R. O. Roça, and R. C. Borra. 2018. Zebrafish assay as a useful model for food security?. In: *V Simpósio Zebrafish como Modelo Animal de Pesquisa,* Universidade Federal do Rio Grande do Norte.

Giacomotto, J., and L. Ségalat. 2010. High-throughput screening and small animal models,

where are we? *British Journal of Pharmacology.* 160(2):204-216. doi:0.1111/j.1476-5381.2010.00725.x

Glynn, A. W., L. Norrgren, and Å Müssener. 1994. Differences in uptake of inorganic mercury and cadmium in the gills of the zebrafish, *Brachydanio rerio*. *Aquatic Toxicology.* 30(1):13-26. doi:10.1016/0166-445X(94)90003-5

Gosslau, A. 2016. Assessment of food toxicology. *Food Science and Human Wellness.* 5(3):103-115. doi:10.1016/j.fshw.2016.05.003

Haendel, M. A., F. Tilton, G. S. Bailey, and R. L. Tanguay. 2004. Developmental toxicity of the dithiocarbamate pesticide sodium metam in zebrafish. *Toxicological Sciences.* 81(2):390-400. doi:10.1093/toxsci/kfh202

Hen Chow, E.S., and S. H. Cheng. 2003. Cadmium affects muscle type development and axon growth in zebrafish embryonic somitogenesis. *Toxicological Sciences.* 73(1):149-159. doi:10.1093/toxsci/kfg046

Hill, A. J., H. Teraoka, W. Heideman, and R. E. Peterson, R.E. 2005. Zebrafish as a model vertebrate for investigating chemical toxicity. *Toxicological Sciences.* 86(1):6-19. doi:10.1093/toxsci/kfi110

Houck, K. A.; and R. J. Kavlock. 2008. Understanding mechanisms of toxicity: insights from drug discovery research. *Toxicology and Applied Pharmacology.* 227(2):163-178. doi:10.1016/j.taap.2007.10.022

Howe, K., M. D. Clark, C. F. Torroja, J. Torrance, C. Berthelot, M. Muffato, and S. Mclaren. 2013. The zebrafish reference genome sequence and its relationship to the human genome. *Nature.* 496(7446):498. doi:10.1038/nature12111

Howitz, K. T., K. J. Bitterman, H. Y. Cohen, D. W. Lamming, S. Lavu, J. G. Wood, and B. Scherer. 2003. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature.* 425(6954):191. doi:10.1038/nature01960

Jagannathan-Bogdan, M.; and L. I. Zon. 2013. Hematopoiesis. *Development.* 140:2463–2467.

Joshi, V., and K. Pancharatna. 2018. Food colorant Sunset Yellow (E110) intervenes developmental profile of zebrafish (*Danio rerio*). *Journal of Applied Toxicology.* 39:571-581. doi:10.1002/jat.3747

Joshi, V., and P. Katti. 2018. Developmental Toxicity Assay for Food Additive Tartrazine Using Zebrafish (*Danio rerio*) Embryo Cultures. *International Journal of Toxicology.* 37(1):38-44. doi:10.1177/1091581817735227

Kari, G., U. Rodeck, and A. P. Dicker. 2007. Zebrafish: an emerging model system for human disease and drug discovery. *Clinical Pharmacology & Therapeutics*, 82(1):70-80. doi:10.1038/sj.clpt.6100223

524 Keshari, V., B. Adeeb, A. E. Simmons, T. W. Simmons, and C. Q. Diep. 2016. Zebrafish as a
525 model to assess the teratogenic potential of nitrite. *Journal of Visualized Experiments: JoVE.*
526 108:1-6. doi:10.3791/53615
527
528 Kim, J. Y., J. Seo, and K. H. Cho. 2011. Aspartame-fed zebrafish exhibit acute deaths with
529 swimming defects and saccharin-fed zebrafish have elevation of cholesteryl ester transfer
530 protein activity in hypercholesterolemia. *Food and Chemical Toxicology.* 49(11):2899-905.
531 doi:10.1016/j.fct.2011.08.001
532
533 Knudsen, T. B., D. A. Keller, M. Sander, E. W. Carney, N. G. Doerrer, D. L. Eaton, and P. B.
534 Watkins. 2015. FutureTox II: in vitro data and in silico models for predictive
535 toxicology. *Toxicological Sciences.* 143(2):256-267. doi:10.1093/toxsci/kfu234
536
537 Kurnianingsih, N., J. P. Utami, N. Nurdiana, and D. Lyrawati. 2016. Monosodium glutamate
538 exposure at early developmental stage increases apoptosis and stereotypic behavior risks on
539 zebrafish (*Danio rerio*) larvae. *Indonesian Journal of Pharmacy.* 27(3):128.
540 doi:10.14499/indonesianjpharm27iss3pp128
541
542 Labrot, F., J. F. Narbonne, P. Ville, M. Saint Denis, and D. Ribera. 1999. Acute toxicity,
543 toxicokinetics, and tissue target of lead and uranium in the clam *Corbicula fluminea* and the
544 worm *Eisenia fetida*: comparison with the fish *Brachydanio rerio. Archives of Environmental*
545 *Contamination and Toxicology.* 36(2):167-178. doi:10.1007/s002449900457
546
547 Lawrence, C. 2016. New frontiers for zebrafish management**.** In: DETRICH III, H. W.,
548 WESTERFIELD, M., ZON L. *Methods in Cell Biology*. Ed. Elsevier, 135(24):483-508,
549 Academic Press. doi:10.1016/bs.mcb.2016.04.015
550
551 Lefebvre, K. A., V. L. Trainer, and N. L. Scholz. 2004. Morphological abnormalities and
552 sensorimotor deficits in larval fish exposed to dissolved saxitoxin. *Aquatic*
553 *Toxicology.* 66(2):159-170. doi:10.1016/j.aquatox.2003.08.006
554
555 Leung, M. C., P. L. Williams, A. Benedetto, C. Au, K. J. Helmcke, M. Aschner, and J. N.
556 Meyer. 2008. *Caenorhabditis elegans*: an emerging model in biomedical and environmental
557 toxicology. *Toxicological Sciences.* 106(1):5-28. doi:10.1093/toxsci/kfn121
558
559 Levin, E. D., E. Chrysanthis, K. Yacisin, and E. Linney. 2003. Chlorpyrifos exposure of
560 developing zebrafish: effects on survival and long-term effects on response latency and spatial
561 discrimination. *Neurotoxicology and Teratology.* 25(1):51-57. doi:0.1016/S0892-
562 0362(02)00322-7
563
564 Li, C., P. Li, Y. M. Tan, S. H. Lam, E. C. Chan, and Z Gong. 2016. Metabolomic
565 characterizations of liver injury caused by acute arsenic toxicity in zebrafish. *Plos*
566 *One.* 11(3):e0151225. doi:10.1371/journal.pone.0151225
567
568 Lieschke, G. J., and P; D; Currie. 2007. Animal models of human disease: zebrafish swim into
569 view. *Nature Reviews Genetics*, 8(5):353. doi:10.1038/nrg2091
570
571 Lin, T., Y. Chen, and W. Chen. 2013. Impact of toxicological properties of sulfonamides on
572 the growth of zebrafish embryos in the water. *Environmental Toxicology and*
573 *Pharmacology.* 36(3):1068-1076. doi:0.1016/j.etap.2013.09.009

Love, D. R., F. B. Pichler, A. A. Dodd, B. R. Copp, and D. R. Greenwood. 2004. Technology for high-throughput screens: the present and future using zebrafish. *Current Opinion in Biotechnology.* 15(6):564-571. doi:10.1016/j.copbio.2004.09.004

Luzio, A., S. M. Monteiro, A. A. Fontaínhas-Fernandes, O. Pinto-Carnide, M. Matos, andA. M. Coimbra. 2013. Copper induced upregulation of apoptosis related genes in zebrafish (*Danio rerio*) gill. *Aquatic Toxicology.* 128:183-189. doi:10.1016/j.aquatox.2012.12.018

Macrae, C.A., and R. T. Peterson. 2015 Zebrafish as tools for drug discovery. *Nature Reviews Drug Discovery.* 14(10):721. doi:10.1038/nrd4627

Makhija, D. T., and A. G. Jagtap. 2014. Studies on sensitivity of zebrafish as a model organism for Parkinson's disease: comparison with rat model. *Journal of Pharmacology & Pharmacotherapeutics.* 5(1):39-46. doi:10.4103/0976-500X.124422

Mcgrath, P., and C. Q. Li. 2008 Zebrafish: a predictive model for assessing drug induced toxicity. *Drug Discovery Today.* 13:394–401. doi:10.1016/j.drudis.2008.03.002

Mezzomo, N. J., B. D. Fontana, A. V. Kalueff, L. J. Barcellos, and D. B. Rosemberg. 2018. Understanding taurine CNS activity using alternative zebrafish models. *Neuroscience & Biobehavioral Reviews.* 83:525-539. doi:10.1016/j.neubiorev.2018.04.012

Mu, X., S. Pang, X. Sun, J. Gao, J. Chen, X. Chen, and C. Wang. 2013. Evaluation of acute and developmental effects of difenoconazole via multiple stage zebrafish assays. *Environmental Pollution.* 175:147-157. doi:10.1016/j.envpol.2012.12.029

OECDILIBRARY. 2019. Accessed in April 04, 2019. http://www.oecd-ilibrary.org/

OECD 210. Fish, Early-life Stage Toxicity Test. 2013. *OECD GUIDELINES FOR THE TESTING OF CHEMICALS.* OECD/OCDE N. 210. Last Modified July 26, 2013. Accessed in April 04, 2019. https://www.oecd-ilibrary.org/docserver/9789264203785-en.pdf?expires=1554216071&id=id&accname=guest&checksum=EF995028174FABC21AF91A98B99E5F97

OECD 236. Fish Embryo Acute Toxicity. 2013. *OECD GUIDELINES FOR THE TESTING OF CHEMICALS.* OECD/OCDE N. 236. Last Modified July 26, 2013. Accessed in April 04, 2019. https://www.oecd-ilibrary.org/docserver/9789264203709-en.pdf?expires=1554216347&id=id&accname=guest&checksum=98B3CA87CA423D51D70FAF3B708EA660

OECD 229. Fish Short-term Reproduction Assay. 2012. *OECD GUIDELINES FOR THE TESTING OF CHEMICALS.* OECD/OCDE N. 229. Last Modified October 2, 2012. Accessed in April 04, 2019. http://www.oecd.org/env/test-no-229-fish-short-term-reproduction-assay-9789264185265-en.htm

OECD 420. Acute Oral Toxicity – Fixed Dose Procedure. 2001. *OECD GUIDELINES FOR THE TESTING OF CHEMICALS.* OECD/OCDE N. 420. Last Modified December 17, 2001. Accessed in April 04, 2019.

https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl420.pdf

Oliveira, R., S. Mcdonough, J. C. Ladewig, A. M. Soares, A. J. Nogueira, and I. Domingues. 2013. Effects of oxytetracycline and amoxicillin on development and biomarkers activities of zebrafish *(Danio rerio). Environmental Toxicology and Pharmacology.* 36(3):903-912. doi:10.1016/j.etap.2013.07.019

Pandey, U. B., and C. D. Nichols. 2011. Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. *Pharmacological Reviews.* 63(2):411-436. doi:10.1124/pr.110.003293

Paustenbach, D. J. 2000. The practice of exposure assessment: a state-of-the-art review. *Journal of Toxicology and Environmental Health Part B: Critical Reviews.* 3(3):179-291. doi:10.1080/10937400050045264

Pereira, T. C. B., M. M. Campos, and M.R. Bogo. 2016. Copper toxicology, oxidative stress and inflammation using zebrafish as experimental model. *Journal of Applied Toxicology.* 36(7):876-885. doi:10.1002/jat.3303

Prussing, K., A. Voigt, and J. B. Schulz. 2013. Drosophila melanogaster as a model organism for Alzheimer's disease. *Molecular Neurodegeneration.* 8(1):35. doi:10.1186/1750-1326-8-35

Raldua, D., and B. Pina. 2014. In vivo zebrafish assays for analyzing drug toxicity. *Expert Opinion on Drug Metabolism & Toxicology.* 10(5):685-697. doi:10.1517/17425255.2014.896339

Ree, F. V. G. E., and J. F. Payne. 1997. Effect of toxaphene on reproduction of fish. *Chemosphere.* 34(4):855-867. doi:0.1016/S0045-6535(97)00016-7

Roex, E. W., R. Keijzers, and C. A. Van Gestel. 2003. Acetylcholinesterase inhibition and increased food consumption rate in the zebrafish, *Danio rerio,* after chronic exposure to parathion. *Aquatic Toxicology.* 64:451–460. doi:10.1016/S0166-445X(03)00100-0

Rougier, F., A. Menudier, C. Bosgiraud, and J. A. Nicolas. 1996. Copper and zinc exposure of zebrafish, *Brachydanio rerio* (Hamilton–Buchaman): effects in experimental listeria infection. *Ecotoxicology and Environmental Safety.* 34(2):134-140. doi:10.1006/eesa.1996.0054

Roy, N. M, B. Carneiro, and J. Ochs. (2016). Glyphosate induces neurotoxicity in zebrafish. *Environmental Toxicology and Pharmacology.* 42:45-54. doi:10.1016/j.etap.2016.01.003

Roy, N. M., J. Ochs, E. Zambrzycka, and A. Anderson. (2016). Glyphosate induces cardiovascular toxicity in *Danio rerio. Environmental Toxicology and Pharmacology.* 46:292-300. doi:0.1016/j.etap.2016.08.010

Samson, J. C., R. Goodridge, F. Olobatuyi, and J. S. Weis. 2001. Delayed effects of embryonic exposure of zebrafish (*Danio rerio*) to methylmercury (MeHg). *Aquatic Toxicology.* 51(4):369-376. doi:10.1016/S0166-445X(00)00128-4

673 Sant'anna, M. C. B., V. De Matas Soares, K. J. Seibt, G. Ghisleni, E. P. Rico, D. B.
674 Rosemberg, and M. R. Bogo. 2011. Iron exposure modifies acetylcholinesterase activity in
675 zebrafish (*Danio rerio*) tissues: distinct susceptibility of tissues to iron overload. *Fish*
676 *Physiology and Biochemistry.* 37(3):573-581. doi:10.1007/s10695-010-9459-7
677
678 Santos, M. 2018. Cientista do Instituto Butantan apresenta as vantagens de usar o zebrafish
679 nas pesquisas científicas. Last Modified July 30, 2018. Accessed April 02, 2019.
680 https://ufal.br/ufal/noticias/2018/7/cientista-do-instituto-butantan-apresenta-as-vantagens-de-
681 usar-o-zebrafish-nas-pesquisas-cientificas
682
683 Shi, X., L. W. Yeung, P. K. Lam, R. S. Wu, and B. Zhou. 2009. Protein profiles in zebrafish
684 (*Danio rerio*) embryos exposed to perfluorooctane sulfonate. *Toxicological*
685 *Sciences.* 110(2):334-340. doi:10.1093/toxsci/kfp111
686
687 Sissener, N. H., L. E. Johannessen, E. M. Hevrøy, C. R. Wiik-Nielsen, K. G. Berdal, A.
688 Nordgreen, and G. I. Hemre. 2010. Zebrafish *(Danio rerio)* as a model for investigating the
689 safety of GM feed ingredients (soya and maize); performance, stress response and uptake of
690 dietary DNA sequences. *British Journal of Nutrition.* 103(1):3-15.
691 doi:10.1017/S0007114509991401
692
693 Simmons, A. E., I. Karimi, M. Talwar, and T. W. Simmons. 2012. Effects of nitrite on
694 development of embryos and early larval stages of the zebrafish (*Danio*
695 *rerio*). *Zebrafish.* 9(4):200-206. doi:10.1089/zeb.2012.0746
696
697 Sulukan, E., M. Köktürk, H. Ceylan, S. Beydemir, M. Işik, M. Atamanalp, and S. B. Ceyhun.
698 2017. An approach to clarify the effect mechanism of glyphosate on body malformations
699 during embryonic development of zebrafish *(Daino rerio). Chemosphere.* 180:77-85.
700 doi:10.1016/j.chemosphere.2017.04.018
701
702 Sun, H., M. Xia, C. P. Austin, and R. Huang. 2012. Paradigm shift in toxicity testing and
703 modeling. *The AAPS Journal.* 14(3):473-480. doi:10.1208/s12248-012-9358-1
704
705 Todd, N. E., and M. Van Leeuwen. 2002. Effects of Sevin (carbaryl insecticide) on early life
706 stages of zebrafish (*Danio rerio*). *Ecotoxicology and Environmental Safety.* 53(2):267-272.
707 doi:10.1006/eesa.2002.2231
708 Troxel, C. M., A. P. Reddy, P. E. O'neal, J. D. Hendricks, and G. S. Bailey. 1997. *In vivo*
709 aflatoxin B1 metabolism and hepatic DNA adduction in Zebrafish (*Danio rerio*). *Toxicology*
710 *and Applied Pharmacology.* 143(1):213-220. doi:0.1006/taap.1996.8058
711
712 Tsay, H. J., Y. H. Wang, W. L. Chen, M. Y. Huang, and Y. H. Chen. 2007. Treatment with
713 sodium benzoate leads to malformation of zebrafish larvae. *Neurotoxicology and Teratology.*
714 29(5):562-569. doi:10.1016/j.ntt.2007.05.001
715
716 Velasco-Santamaría, Y. M., R. D. Handy, and K. A. Sloman. 2011. Endosulfan affects health
717 variables in adult zebrafish (*Danio rerio*) and induces alterations in larvae
718 development. *Comparative Biochemistry and Physiology Part C: Toxicology &*
719 *Pharmacology.* 153(4):372-380. doi:0.1016/j.cbpc.2011.01.001
720
721 Zhang, Y., X. Wang, X. Yin, M. Shi, R. A. Dahlgren, and H. Wang. 2016. Toxicity
722 assessment of combined fluoroquinolone and tetracycline exposure in zebrafish *(Danio*

rerio). Environmental Toxicology. 31(6):736-750. doi:10.1002/tox.22087

Zhang, Q., J. Cheng, and Q. Xin. 2015. Effects of tetracycline on developmental toxicity and molecular responses in zebrafish *(Danio rerio)* embryos. *Ecotoxicology.* 24(4):707-719. doi:10.1007/s10646-015-1417-9

Zhang, W., X. Sun, L. Chen, K. F. Lin, Q. X. Dong, C. J. Huang, and J. Zhu. 2012. Toxicological effect of joint cadmium selenium quantum dots and copper ion exposure on zebrafish. *Environmental Toxicology and Chemistry.* 31(9):2117-2123. doi:10.1002/etc.1918

Zhang, J., J. Meng, Y. Li, and C. Hu. 2010. Investigation of the toxic functional group of cephalosporins by zebrafish embryo toxicity test. *Archiv der Pharmazie.* 343(10):553-560. doi:10.1002/ardp.201000005

Zhang, C., C. Willett, and T. Fremgen. 2003. Zebrafish: an animal model for toxicological studies. *Current Protocols in Toxicology.* 17(1):1-7. doi:10.1002/0471140856.tx0107s17

Zorzetto, R., and M. Guimarães. 2013. Um peixe modelo. *Pesquisa FAPESP.* 209:16-21. Last Modified July, 2013. Accessed Apil 02, 2019. http://revistapesquisa.fapesp.br/wp-content/uploads/2013/07/016-021_CAPA_cobaias_209.pdf

Weerasooriyagedara, M. S. 2018. Toxicity Effects of Aspartame on Embryonic Development of Zebrafish (*Danio Rerio*). *International Journal of Engineering and Management Research (IJEMR).* 8(1):183-188.

Wiegand, C., E. Krause, C. Steinberg, and S. Pflugmacher. 2001. Toxicokinetics of atrazine in embryos of the zebrafish (*Danio rerio*). *Ecotoxicology and Environmental Safety.* 49(3):199-205. doi:10.1006/eesa.2001.2073

Willey, J. B., and P. H. Krone. 2001. Effects of endosulfan and nonylphenol on the primordial germ cell population in pre-larval zebrafish embryos. *Aquatic Toxicology.* 54(1-2):113-123. doi:10.1016/S0166-445X(00)00178-8

Wu, T. S., J. J. Yang, F. Y. Yu, and B. H. Iu. 2012. Evaluation of nephrotoxic effects of mycotoxins, citrinin and patulin, on zebrafish (*Danio rerio*) embryos. *Food and Chemical Toxicology.* 50(12):4398-4404. doi:10.1016/j.fct.2012.07.040

Table 1. Comparison of animal models used in human health research

Attribute of animal model	Model organism				
	Fly	**Zebrafish**	**Mouse**	**Rat**	**Authors**
Practical Issues					
Husbandry infrastructure	$	$	$$$	$$$	Lieschke and Currie (2007)
Cost per animal per year	$	$	$$$	$$$	Lieschke and Currie (2007)
Daily cost	-	U$ 0.16	-	U$ 2.15	Santos (2018)
Body plane	Invertebrate	Vertebrate	Vertebrate	Vertebrate	Zorzetto and Guimarães (2013)
Sexual maturation	20 days	60-90 days	85 days	-	Zorzetto and Guimarães (2013)
Breeding production	100 eggs/day	100 eggs/day	10 descendants/2 months	-	Zorzetto and Guimarães (2013)
Characterized inbred strains	+	-	++++	+++	Lieschke and Currie (2007)
Outbred laboratory strains	+	+++	++	++	Lieschke and Currie (2007)
Anatomical similarity	-	+	++	++	Lieschke and Currie (2007)
Molecular or genetic similarity	+	++	+++	+++	Lieschke and Currie (2007)
Genetic similarity	60%	70%	85%	-	Zorzetto and Guimarães (2013) Howe et al. (2013)
Pathological similarity	-	++	+++	+++	Lieschke and Currie (2007)
Transparency of embryos	No	Yes	No	No	Zorzetto and Guimarães (2013)
Fecundation	Internal	External	Internal	Internal	Zorzetto and Guimarães (2013)
Development of embryos	External	External	Internal	Internal	Zorzetto and Guimarães (2013)
Storage; for example, freezing sperm	No	Yes	Yes	Yes	Lieschke and Currie (2007)
Molecular biology tools					
Transgenesis*	++	++	++	++	Lieschke and Currie (2007)
Targeted gene modification*	+	-	++++	+	Lieschke and Currie (2007)
Transient *in vivo* assays*	++	++++	+	+	Lieschke and Currie (2007)
Allelic series from TILLING*	+++	++++	++	+	Lieschke and Currie (2007)
Feasibility of large-scale screens‡	++++	+++	++	+	Lieschke and Currie (2007)
Affordability of large-scale screens‡	++++	+++	+	-	Lieschke and Currie (2007)
Sequencing progress§	+++	++	+++	++	Lieschke and Currie (2007)
Annotation progress§	++	++	++++	++	Lieschke and Currie (2007)
Cell-biology tools					
Cell lines and tissue culture	++	+	++++	+	Lieschke and Currie (2007)
Antibody reagentes	++	+	++++	++	Lieschke and Currie (2007)

*Reverse-genetics approach; ‡forward-genetics approach; §genome sequence; -, not relevant, or not a strength; $, $$, $$$ and +, ++, +++, relative cost ($) and strength (+) of the model in each category; ++++, outstanding strength of the model; TILLING, targeting induced local lesions in genomes.

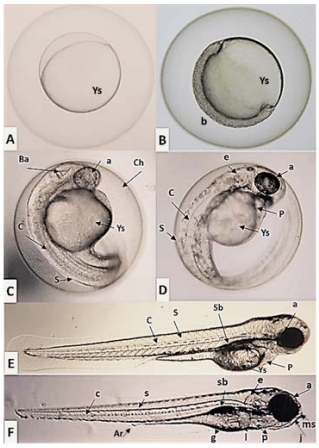

Figure 1. Zebrafish *Danio rerio* embryo (A) 0 hour post fertilization (h.p.f.); (B) 5 h.p.f.; (C) 24 h.p.f.; (D) 48 h.p.f.; (E) 72h.p.f.; (F) 96h.p.f; (Ys) yolk sac; (b) blástula; (a) eye anlage; (Ba) brain anlage; (ch) chorion; (c) chorda; (s) somites; (e) ear; (p) pericard; (Sb) swimming bladder; (l) liver; (g) gut; (ms) mouth slit; (j) jaw; (Ar) anal region.

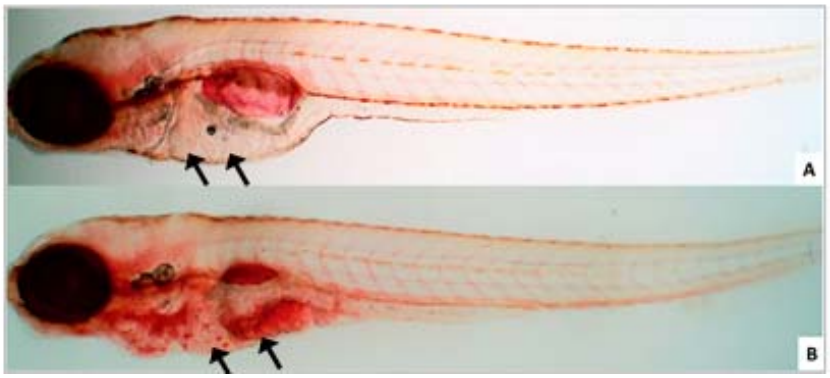

Figure 2. *Danio rerio* 120h.p.f. stained with Oil red O (sigma): (A) Negative control, arrows indicate absence of red coloration; (B) Positive control (ethanol 2%), arrows indicate hepatic

steatosis and yolk sac retention.

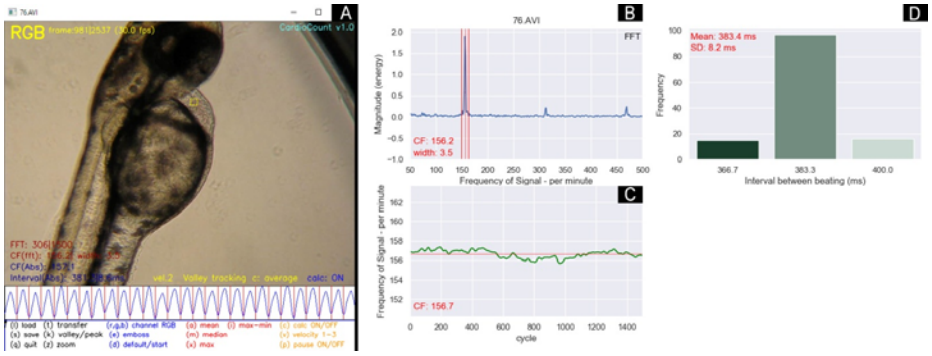

Figure 3. Screens of the Cardio Count Software v1.0 (ZEBRA Advances, UFSCAR– São Carlos, Brazil) showing (A) a video of larva (90 s with 2442 frames at 30 frames per second) with the region of interest (ROI) located manually by user over the heart in activity (yellow square) and the graphic showing the cycles of beating (contraction / relaxion) calculated from ROI. In right, it is possible to observe three graphics generated by software during the analysis. In (B) it is showed the power spectrum of analysis of Fast Fourie Transform (FFT) calculated from variation of mean color of ROI. In this graphic the cardiac frequency (CF) and the width of the band (WIDTH) are showed in red. In (C) it is showed the variation of the rate beating (per min) during the cycles of heart activity with highlight of the cardiac frequency calculated by other method (peaks analysis) than FFT. The (D) graphic shows the distribution of the intervals between heart beating (in millisecond), highlighting the MEAN and standard deviation (SD), which can be used to analysis arrhythmias. The graphics are generated during the analysis in real time and it are used as individual parameters of cardiac activity of each larvae / embryo. The software, wrote in Python, calculates the cardiac parameters by Fast Fourier Transform and peak analysis of color variation profile from ROI (under publication).