



Zebrafish early-life stage as a refinement to predict acute oral toxicity

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Introduction

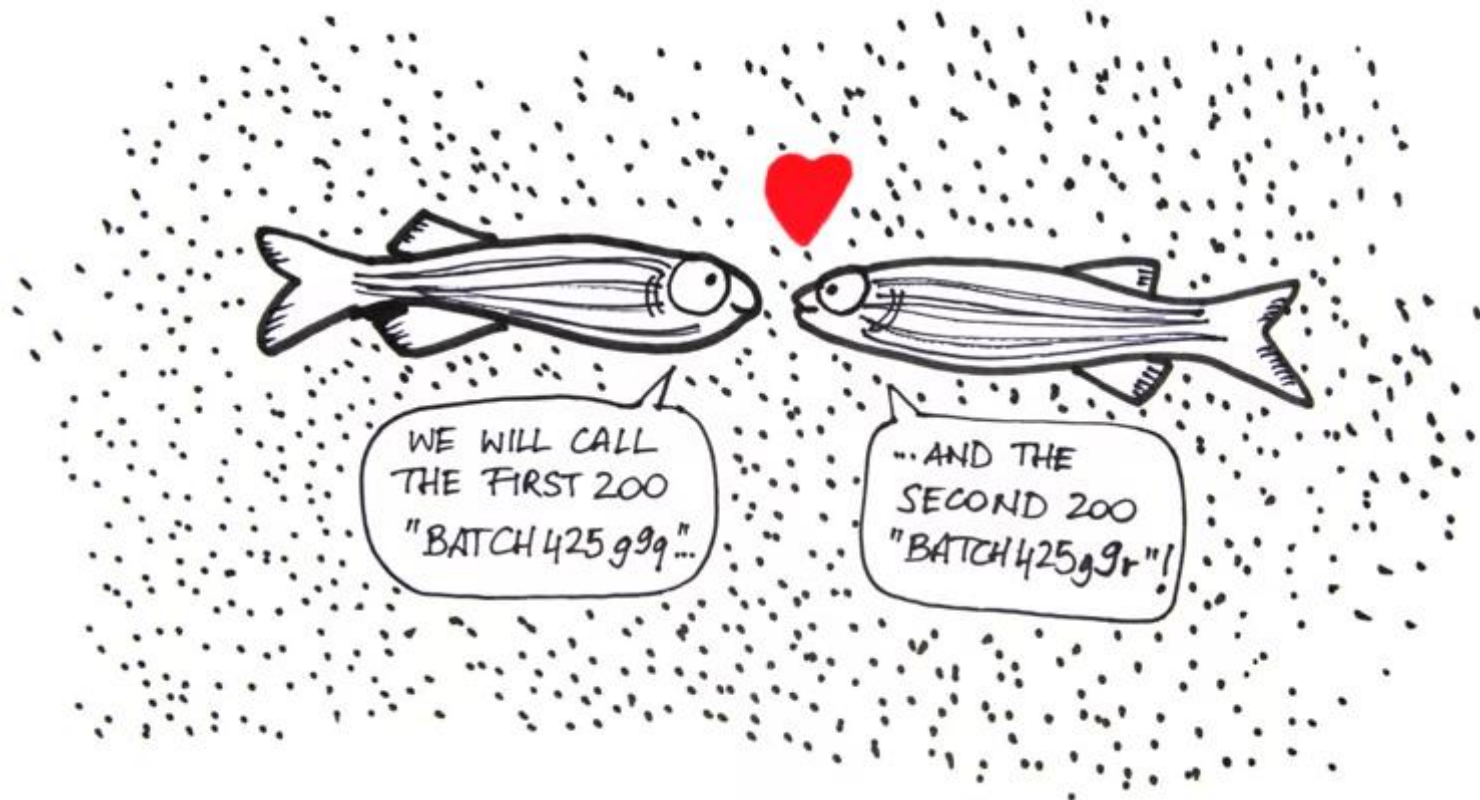
- ✓ Zebrafish (*Danio rerio*) is originally from **South East Asia** (Bangladesh, India, Nepal, Thailand).
- ✓ Found in sweet and brackish waters;
- ✓ They are undemanding and thrive in **almost all conditions**.





Introduction

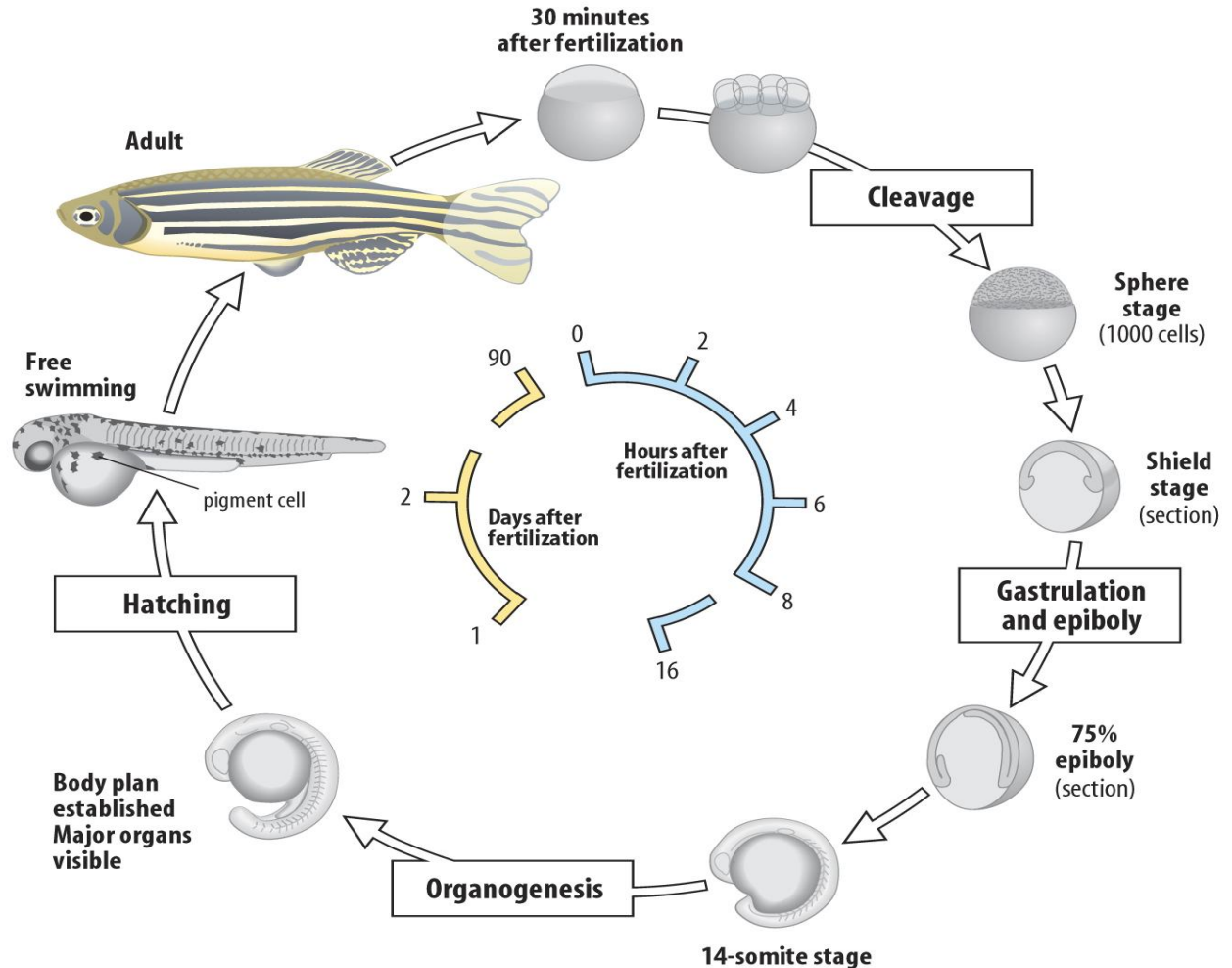
- ✓ They show a **good reproduction** in captivity;
- ✓ Each female generates about **two hundred eggs**.





Introduction

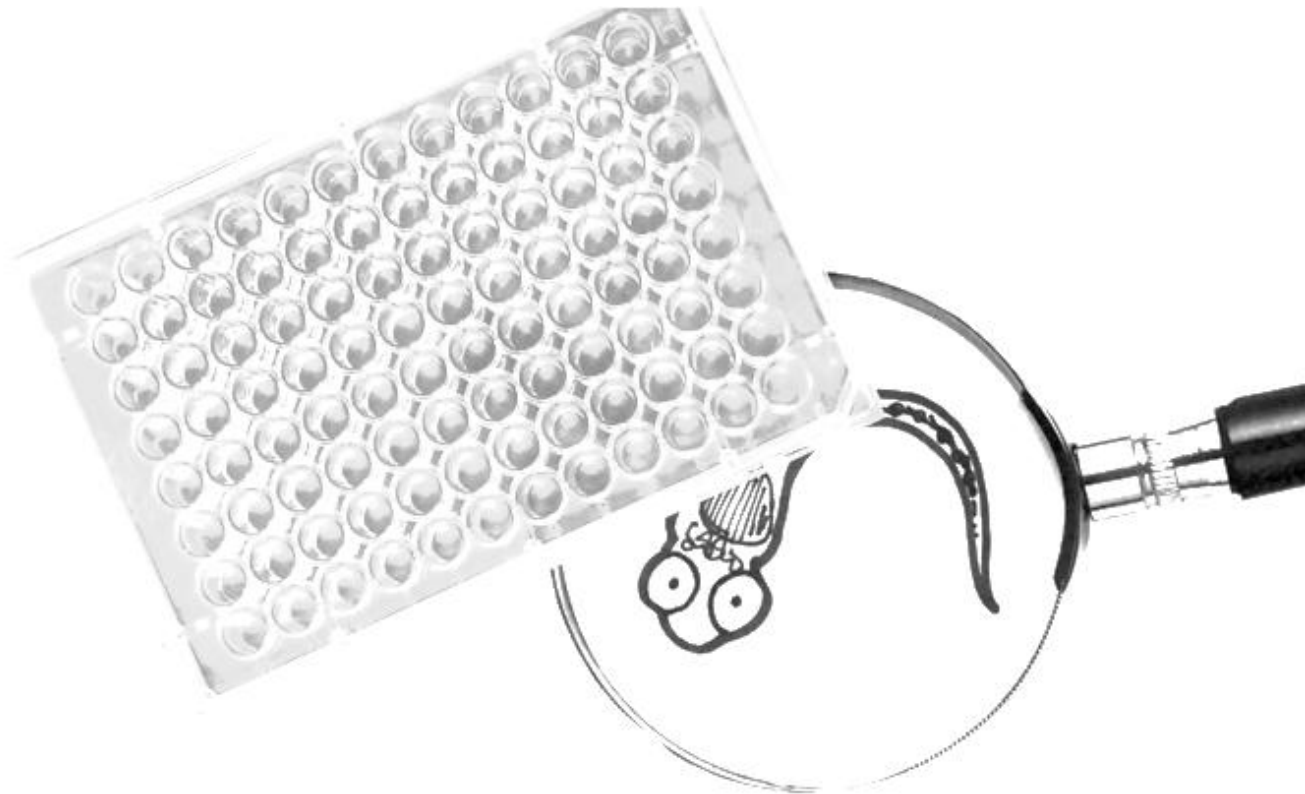
- ✓ Short life cycle;
- ✓ The eggs hatch between **two to three days**, to give zebrafish **larvae**.





Introduction

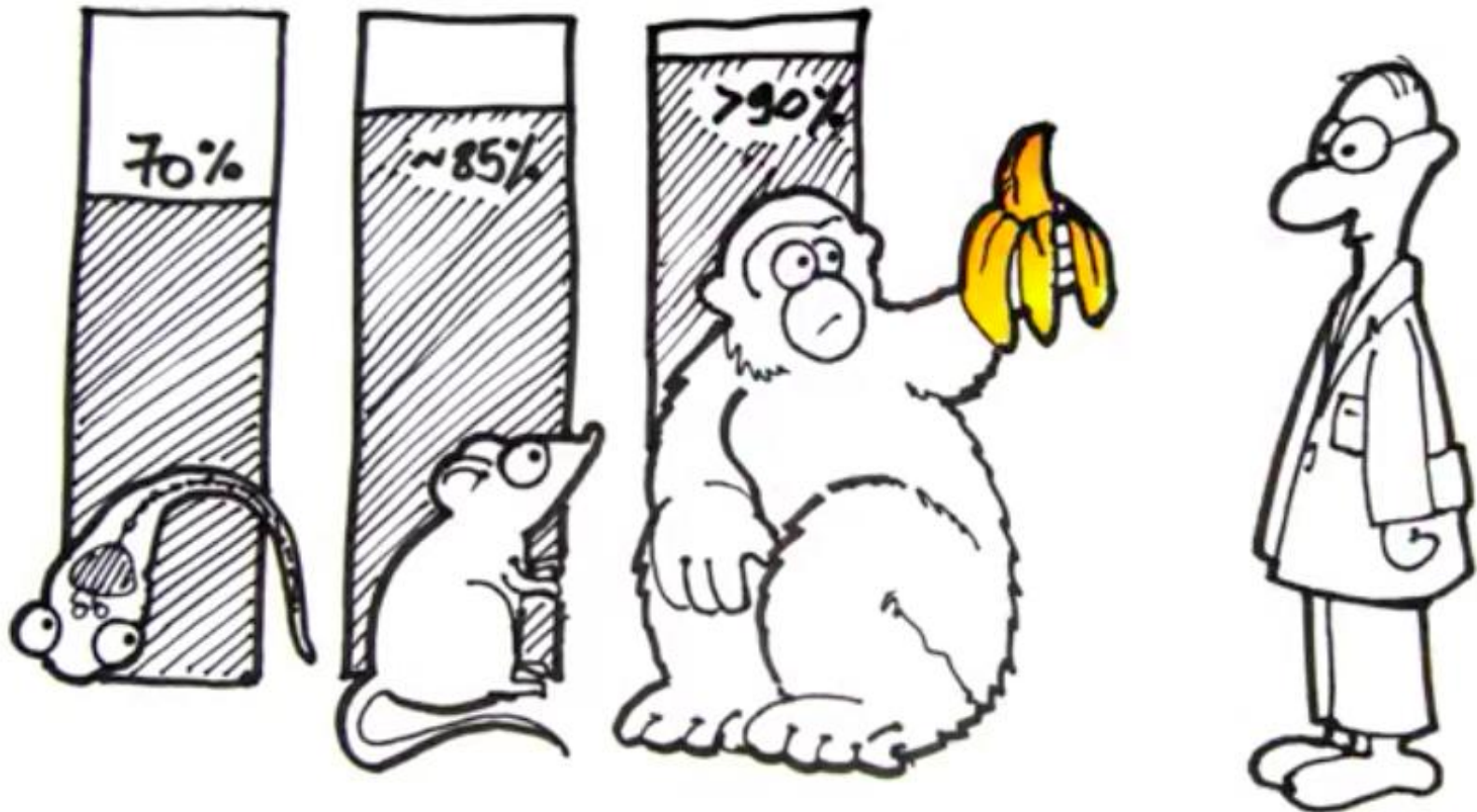
- ✓ Technically, they are not considered laboratory animals, until five days post-fertilization, because they do **not feed and are free from pain.**





Introduction

- ✓ They present a genetic similarity of 70% with humans, being less than mouse (85%) and chimpanzees (90%).





Introduction

- ✓ Besides that, zebrafish embryonic development is **very similar** to the embryogenesis of higher vertebrates, including human;



FISH



CHICKEN



PIG



HUMAN

"IT'S A GIRL!"

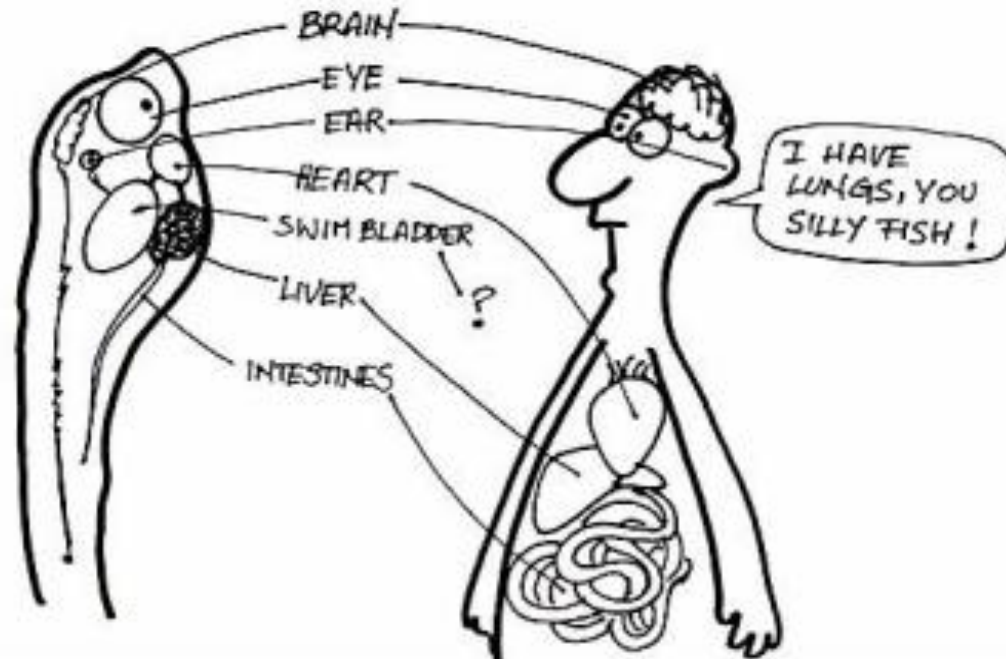


- ✓ it is **very fast** (grow its organs 20 times faster than humans) and can be **easily monitored** through the **transparent egg**.



Introduction

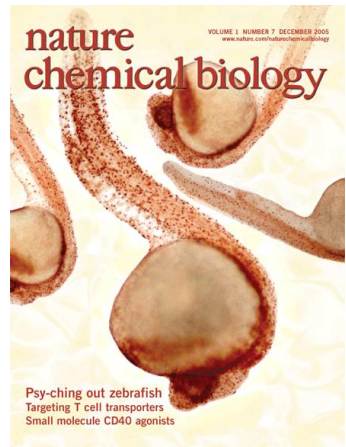
- ✓ Instead of testing potential drugs for safety or efficacy on cultured cells, zebrafish offers those cells within an working organ, and that organ can interact or communicate with the other organs around it.
- ✓ Presenting an multi-organ culture model with a similar body plan and functional structure as a human being.



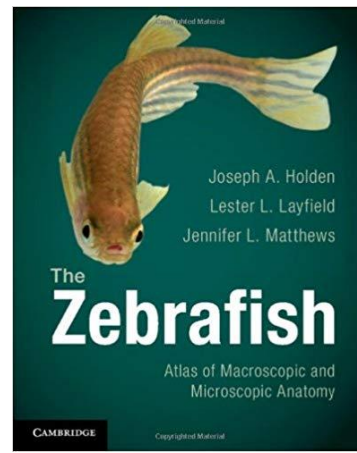
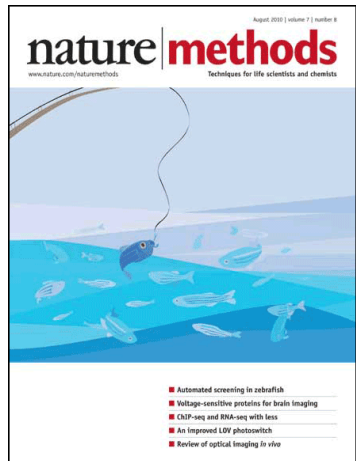
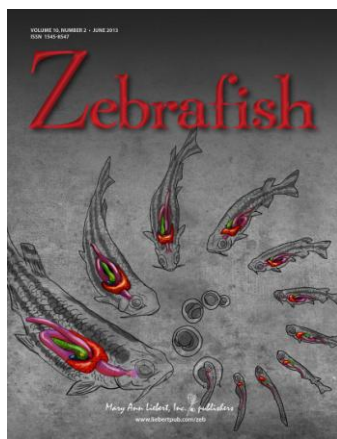
<http://www.youtube.com/watch?v=YO-LUC29zmk>



Introduction



✓ The field of zebrafish research is exploding, since it presents many possibilities for both efficacy and safety testing.





Purpose

Evaluate the acute toxicity of substances with different GHS categories using zebrafish early-life stage to determine LC50 values and compare with *in vivo* acute oral toxicity data from literature



Materials

Category 2

Mercury II chloride

Category 4

Acetylsalicylic acid

Atropine sulfate

Propanolol

Sodium oxalate

Category 3

Cadmium II chloride

Verapamil HCl

Category 5

2-propanol

Acetaminophen

Ethanol

Glycerol

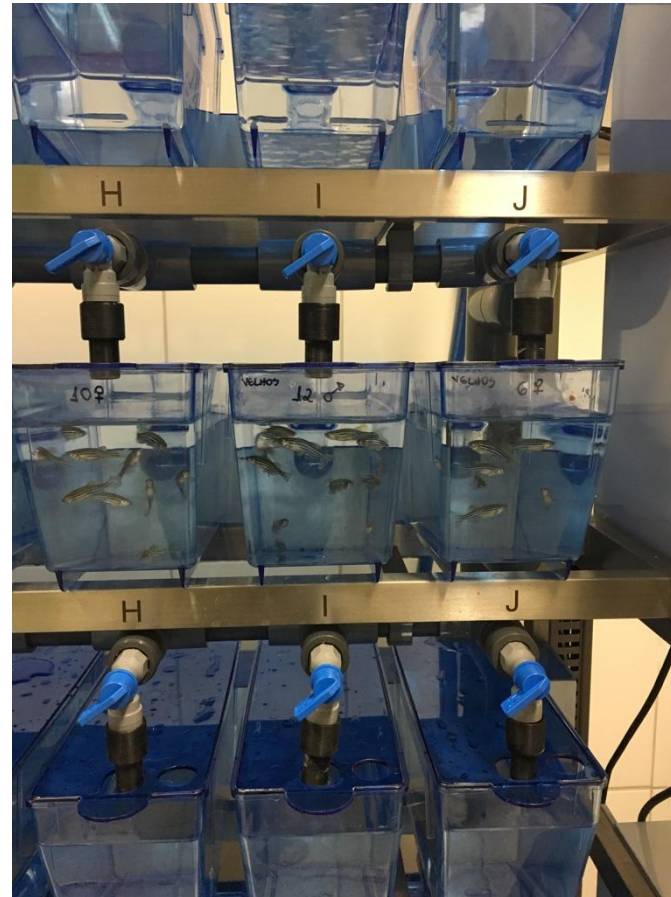
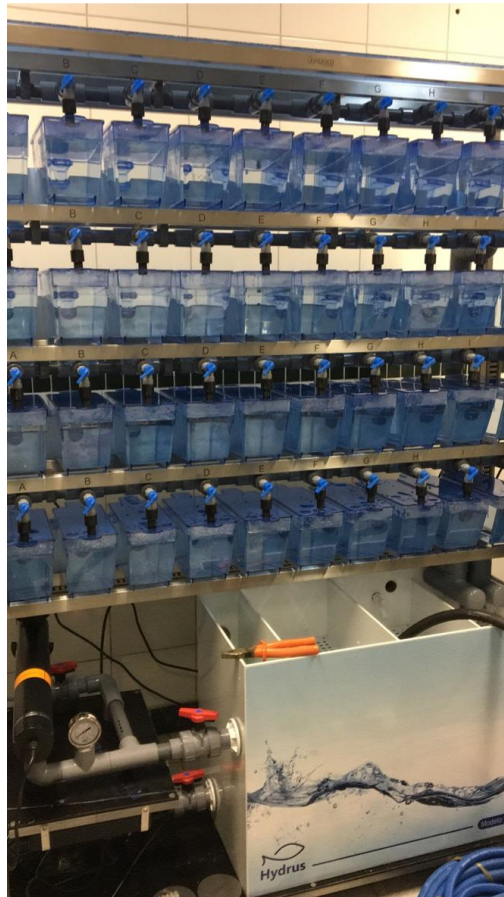
Sodium chloride

Xylene

✓ **13 substances** with different GHS category



Methodology



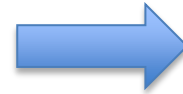
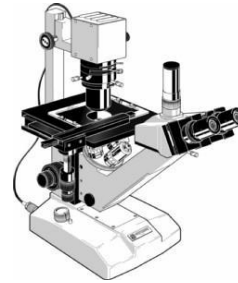
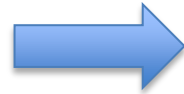
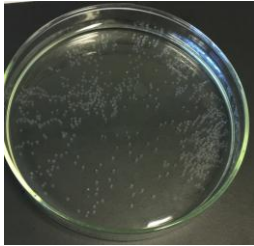
- ✓ Fish were maintained in a Rack Hydrus (Alesco®) recirculating system
- ✓ The assay was performed following OECD 236 guideline



Methodology

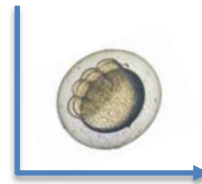


Zebrafish embryos rinsed in water



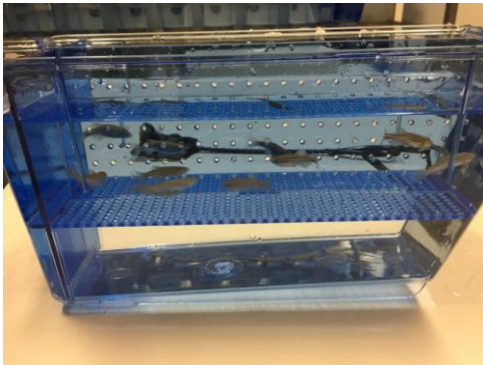
Unfertilized or damaged eggs were discarded

Examined under a stereomicroscope

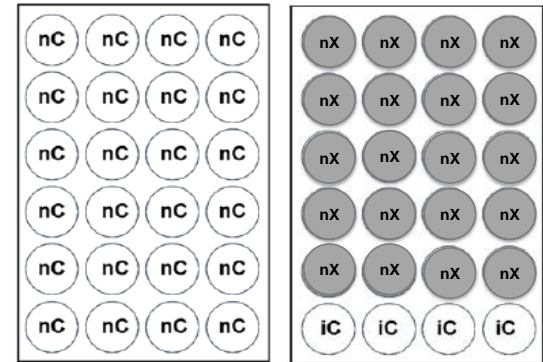


Fertilization rate of 90% were used

Zebrafish eggs were collected about 30 min after natural mating



Male and female of zebrafish (*D. rerio*) 2:1



- ✓ 24 embryos for negative control (nC);
- ✓ 20 embryos for each concentration (nX) of substance test;
- ✓ Tests were performed in triplicate in a climate chamber at 27±1°C and 12h light.
 - ✓ Until 96 h.



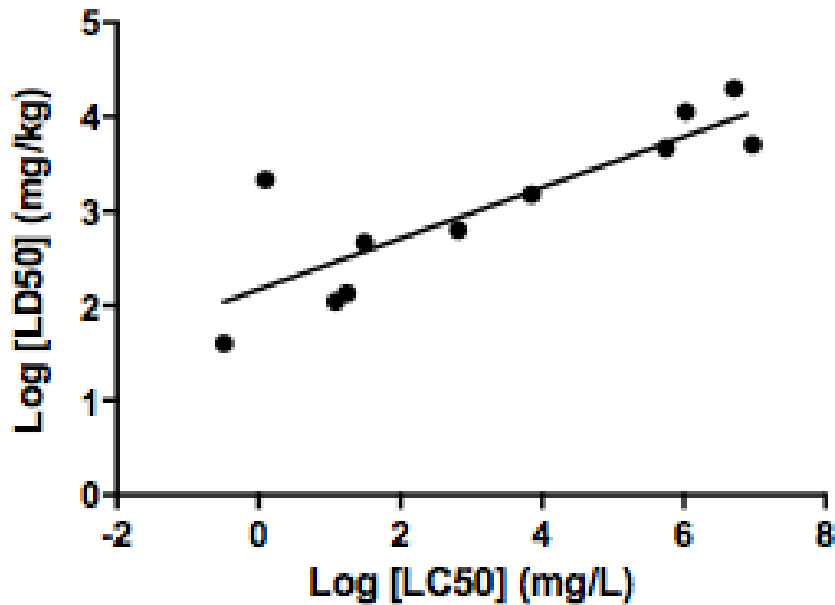
Results

Substance	LC50 (mg/L)	LD50 (mg/kg)(literature)	GHS
Mercury II chloride	0.3266	40	Category 2
Cadmium II chloride	17.67	135	Category 3
Verapamil HCl	12.22	111	Category 3
Acetylsalicylic acid	7.085	1506	Category 4
Atropine sulfate	*	819	Category 4
Propranolol	31.50	466	Category 4
Sodium Oxalate	660.40	633	Category 4
2-propanol	9401070	5105	Category 5
Acetaminophen	1.25	2163	Category 5
Ethanol	1076407	11324	Category 5
Glycerol	5144746	19770	Category 5
Sodium chloride	*	4046	Category 5
Xylene	561461	4667	Category 5



Results

Linear regression



- ✓ A linear regression-model using the values log-transformed was generated for the prediction of LD_{50} from LC_{50} values.

$$\text{Log [LD50]} = 0.2694 \times \text{log[LC50]} + 2.173$$

$$R^2 = 0.72272$$



Results



Chemico-Biological Interactions 277 (2017) 185–194



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journal homepage: www.elsevier.com/locate/chembioint



In vitro genotoxicity and *in vivo* subchronic evaluation of the anti-inflammatory pyrazole compound **LQFM021**



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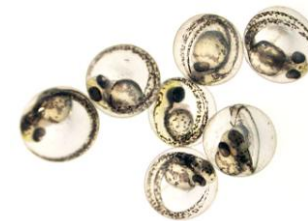
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^e Departamento de Estomatologia (Patologia Oral), Faculdade de Odontologia, Universidade Federal de Goiás, Goiânia, GO, Brazil

✓ The correlation
was confirmed
testing LQFM021



Results



Chemico-Biological Interactions 277 (2017) 185–194



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In vitro gen
inflammato

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3.3. Zebrafish embryo-larval toxicity test

The subchronic effects of different concentrations of LQFM021 (1–100 mg/L) in embryo-larval stages of zebrafish were investigated during 7 dpf (or 168 hpf) of exposure. The lowest concentrations (1–15.8 mg/mL) of LQFM021 did not induce significant lethal or sublethal effects on zebrafish early-life stage during 7 dpf of exposure (data not shown), thus it seems to have no embryotoxic potential in this concentration range. However, the highest concentrations of LQFM021 caused significant malformations in zebrafish larvae from 72 hpf (100 mg/L) and 144 hpf (39.8 mg/L) (Fig. 6A). As a consequence, a significant decrease in larvae survival rate was observed after 120 hpf of exposure to 100 mg/L LQFM021 ($p < 0.001$) (Fig. 6B). Moreover, these higher concentrations induced a significant reduction in **heartbeat rate** of zebrafish larvae at 72 hpf ($p < 0.0001$), when compared to control (Fig. 6C). The main sublethal effects observed in larvae stage were the following: **cardiac edema** ($EC_{50-96\text{ h}} = 71.30\text{ mg/L}$) (Fig. 7B), delayed yolk sac absorption ($EC_{50-96\text{ h}} = 77.70\text{ mg/L}$) (Fig. 7D) and **tail deformity** ($EC_{50-96\text{ h}} > 100\text{ mg/L}$) (Fig. 7E).

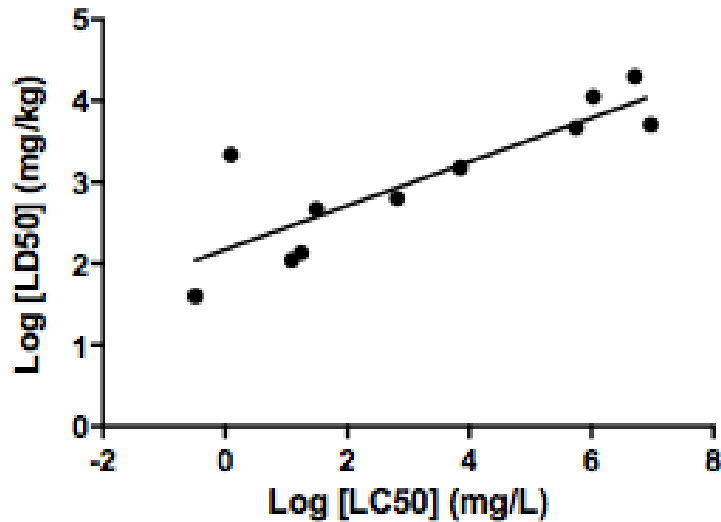


S.



Results

Linear regression



LQFM021
✓ LC50 = 203.2 mg/L;
LD50 (equation) = 623.38 mg/kg → category 4

$$\text{Log [LD50]} = 0.2694 \times \text{log[LC50]} + 2.173$$
$$R^2 = 0.72272$$



Results



524

Regular Article

Chem. Pharm. Bull. 61(5) 524–531 (2013)

Vol. 61, No. 5

Synthesis, Docking Studies, Pharmacological Activity and Toxicity of a Novel Pyrazole Derivative (LQFM 021)—Possible Effects on Phosphodiesterase

Daniella Ramos Martins,^a Francine Pazini,^b Vinícius de Medeiros Alves,^a
Soraya Santana de Moura,^a Luciano Moraes Lião,^b Mariana Torquato Quezado de Magalhães,^c
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Results

Acute Oral Toxicity Evaluation of LQFM 021 Some signs of toxicity in animals after the administration of 2000 mg/kg of LQFM 021 were observed in the present study. In the first four hours, they presented apathy and subsequent paresis in the hind legs. One showed a higher motor impairment and died soon after. The others recovered completely after 24 h. After 14 d, no other signs of abnormality were presented until the animals were euthanized. Therefore, LQFM 021 was categorized as “Class 4” using the Global Harmonized System (GHS) (*i.e.*, acute lethal toxicity between 300 and 2000 mg/kg). There were no significant differences in body weight (data not shown).

pl. 61, No. 5

Considering acute oral toxicity assessment in swiss female mice, it was found the LD50 between 300 to 2000 mg/kg wich classified as GHS category 4.



Conclusion



- Our results suggested that zebrafish embryo can be at least a refinement in the sense of the 3 R's principle to predict acute oral toxicity, being a new alternative method tool for systemic toxicology evaluation.



Collaborators



Laboratório de Pesquisa em
Métodos Alternativos

FF-UFG



Medical
pharmaceutical
chemistry Laboratory
(LQFM-UFG)

Support financial



R

THANKS FOR YOUR ATTENTION!

