Effects of propranolol and haloperidol on non-target organisms: A case study with amphibians

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Effects of propranolol and haloperidol on non-target organisms:
A case study with amphibians

In vivo

Xenopus laevis

Pelophylax perezi

In vitro

A6 cell line

XTC-2 cell line

Amphibians
- Embryos
  - Mortality
  - Malformations
  - Growth
- Tadopoles
  - Mortality
  - Malformations
  - Growth

Amphibian cell lines
- Cell viability
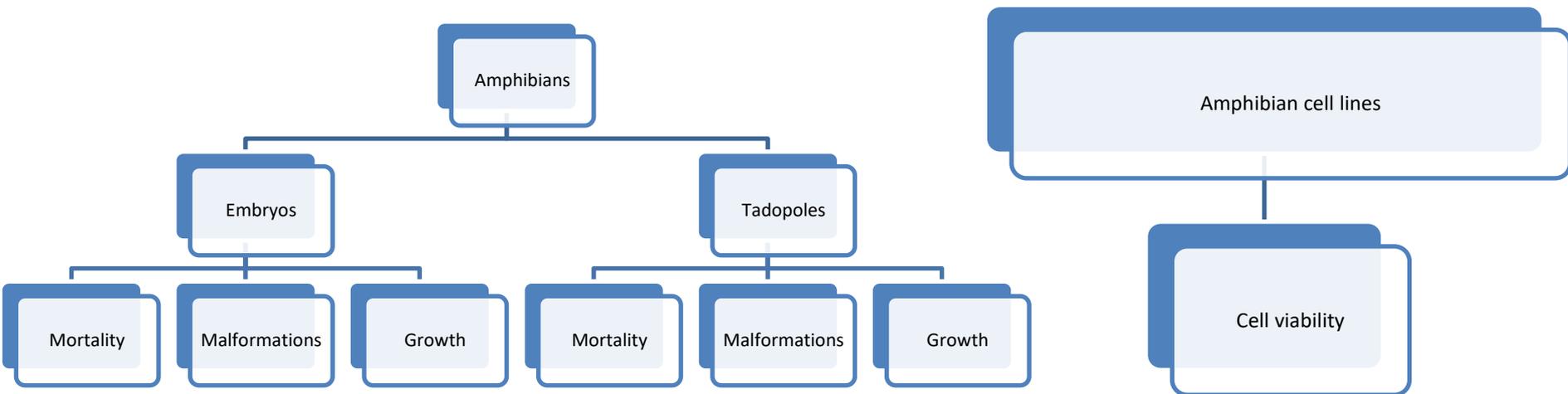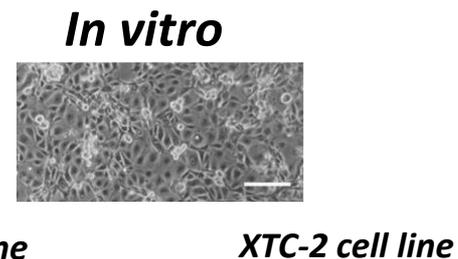
Abstract: The technological expansion allowed the development of active substances that promoted an improvement of human life quality and a population increase. However, the increase in the consumption of pharmaceutical substances, in terms of quantity and variety, has promoted its environmental presence, namely in aquatic systems. Considering the bioactive nature of these substances, undesirable effects may be expected in the biota inhabiting ecosystems that receive these chemicals. Among these organisms are amphibians, that show high sensitivity to environmental changes. The present study aimed to assess the individual effects of two pharmaceutical drugs (propranolol and haloperidol) to aquatic early life stages (embryos and tadpoles) of *Xenopus laevis* and *Pelophylax perezi*. Assessed endpoints include mortality, malformations and growth. Furthermore, in vitro assays, where cell viability was assessed, were performed, to validate its relevance as a non-animal alternative to assess potential risks to amphibians. Thus, embryos and tadpoles of *X. laevis* and *P. perezi* and two cell lines (A6, an epithelial line derived from the kidney of an adult male; and XTC-2, a fibroblast-like line derived from a tadpole) of *X. laevis* were exposed to a range of concentrations of each pharmaceutical alone. Overall, the results showed that *X. laevis* and *P. perezi* tadpoles were more sensitive than the respective embryos to both pharmaceuticals drugs. When comparing the two species, *X. laevis* tended to be more sensitive than *P. perezi*. Furthermore, the in vitro assays were less sensitive to the pharmaceuticals, when compared to the tadpoles' assays.

Keywords: Amphibian cell lines; Aquatic life stages; Ecotoxicity; *Pelophylax perezi*; *Xenopus laevis*

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Introduction

<u>Haloperidol</u>

- Butyrophenone-derivative antipsychotic drug;

- Haloperidol acts as an antagonist on the dopamine (D2) receptor in the central nervous system;

- Approved by the U.S. Food and Drug Administration in 1976 (Lin et al., 2019), to prevent surgical shock;

- Has been applied in the treatment of
  - schizophrenia and in cases of Tourette disorder (Lin et al., 2019)
  - symptoms of autism
  - in hospital emergency settings in cases of nausea, vomiting and abdominal pain (Shahsavari et al., 2021)

- Is still one of the most commonly prescribed antipsychotic drugs (Adams et al., 2013);

- Reported side effects include tremors, muscle stiffness and uncontrollable shaking;

Introduction

<u>Haloperidol</u>

- Has been reported in a sewage treatment plant (Gothenburg, Sweden) at a concentration of 374 ng L^{-1};

- In the Pacific Ocean (near San Francisco, USA) it was detected at a maximum concentration of 56 ng L^{-1} (Nödler et al., 2014);

- Has been detected in the blood plasma of fish (*Oncorhynchus mykiss*) present in the sewage effluent, at a concentration of 1.2 ng mL^{-1};

- The dopamine receptor in which haloperidol acts is evolutionary conserved in various species (Gunnarsson et al., 2008). However, its effects on non-target organisms is not well known.

Introduction

<u>Propranolol</u>

- A nonselective β-blocker that blocks the action of catecholamines (adrenaline and noradrenaline) at both beta-1 and beta-2 adrenergic receptors;

- Has been used in the treatment of cardiovascular diseases such as hypertension (Brunton et al., 2018), coronary artery disease (Peixoto et al., 2020) and congestive heart failure (Brunton et al., 2018);

- Has also been used in prophylaxis treatment of migraine (Brunton et al., 2018), treatment of essential tremor (Al-Majed et al., 2017), anxiety related to public performing (Dowd et al., 2007), and post-traumatic stress disorder (Dowd et al., 2007);

- It has recently been suggested that propranolol can be used in the treatment of cancer, preventing disease progression and metastases, as well as changing the tumour microenvironment;

Introduction

<u>Propranolol</u>

- Competes with agonists such as catecholamines, epinephrine and norepinephrine, for beta receptor sites (Dowd et al., 2007), therefore inhibiting sympathetic effects;

- Propranolol can cross the blood-brain barrier allowing it to act on central nervous system (Steenen et al., 2016);

- Propranolol is among the pharmaceuticals most commonly detected in aquatic environments (Chavoshani et al., 2020);

- β-receptors are present not only on human tissues but also in other animals, thus beta blockers may affect a wide range of species once released in the environment;

- Propranolol has been reported to decreased heart rate and hatching rate in zebrafish larvae (Sun et al., 2014);

- Effects on organisms like amphibians are not well known.

Introduction

Amphibian population and their ecological importance

- Amphibian species are facing the threat of extinction (40% of species; IUCN, 2020);

- Amphibians play a central role in the freshwater ecosystems, being both prey and predators;

- Aquatic life stages of amphibians are very sensitive to chemical exposure (e.g., permeable skin, anamniotic);

- There is a need to determine if *in vitro* methodology could be used as an adequate surrogate for animal experimentation in the risk assessment of contaminants to early aquatic life stages of amphibians.

Introduction

What was tested in this study?

- The toxicity of propranolol and haloperidol was assessed in early life stages (embryos and tadpoles) of two amphibian species *Pelophylax perezi and Xenopus laevis* that have been previously demonstrated good biological models for biomedical studies;

- The cytotoxicity of the pharmaceuticals was assessed on two amphibian established cell lines
    - A6 – Adult male kidney derived epithelial cell line
    - XTC-2 – Tadpole derived fibroblastic cell line

Results and Discussion

Propranolol – *X. laevis*

**Embryos Survival**

96h LC$_{50}$ - 8.10 mg/L
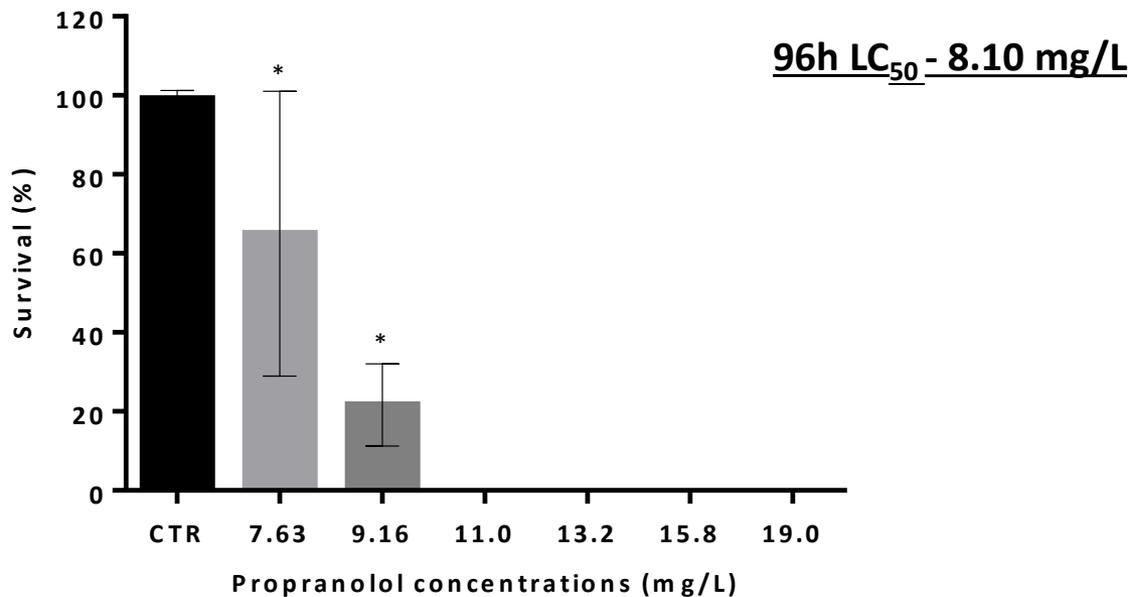
Average survival percentage of *Xenopus laevis* embryos after 96h exposure to propranolol. Error bars represent standard deviation. * indicates significant differences relatively to the control (p<0.001).

Results and Discussion

Propranolol - *X. laevis*

Embryos Malformations
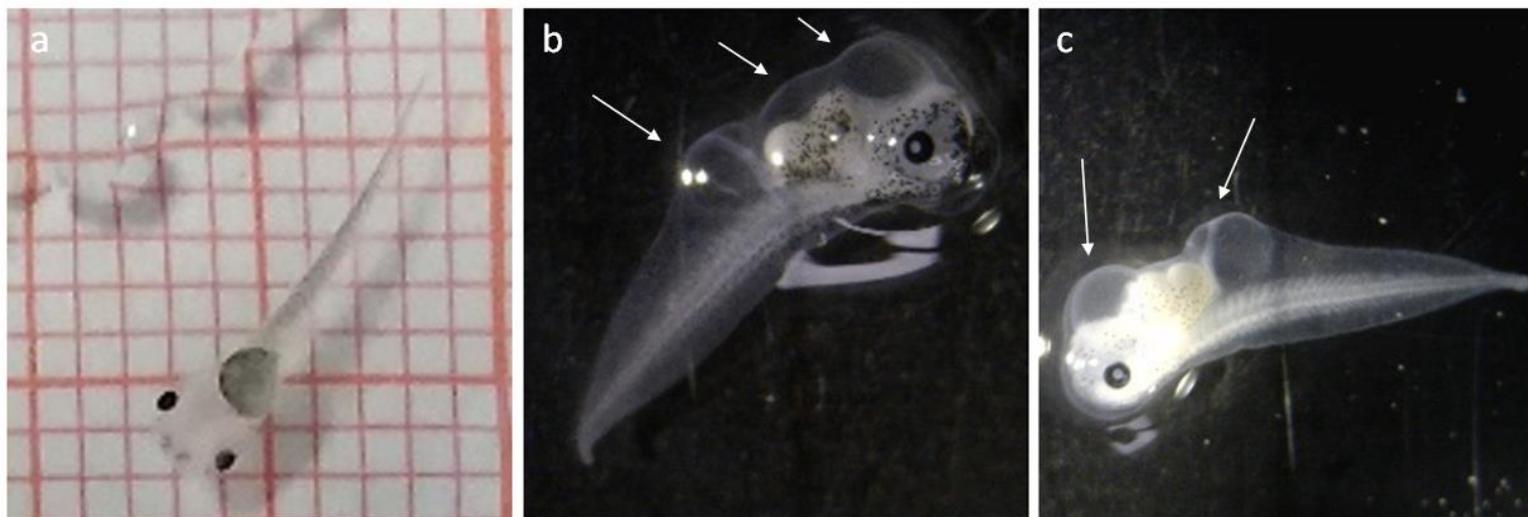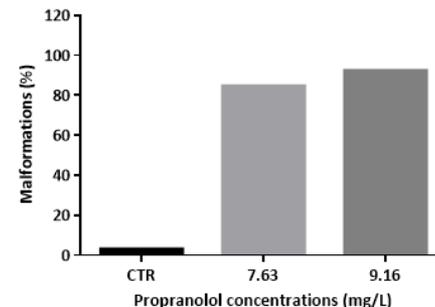
Examples of morphological effects on *Xenopus laevis* at the end of the embryo teratogenicity assay.
(a) Control larva;
(b) Larva exposed to 7.63 mg/L propranolol with severe malformations including heart and proctodaeu edema;
(c) Larvae exposed to 9.16 mg/L propranolol with heart and proctodaeu edema and hypopigmentation

Results and Discussion

Propranolol - *X. laevis*

Effects on growth
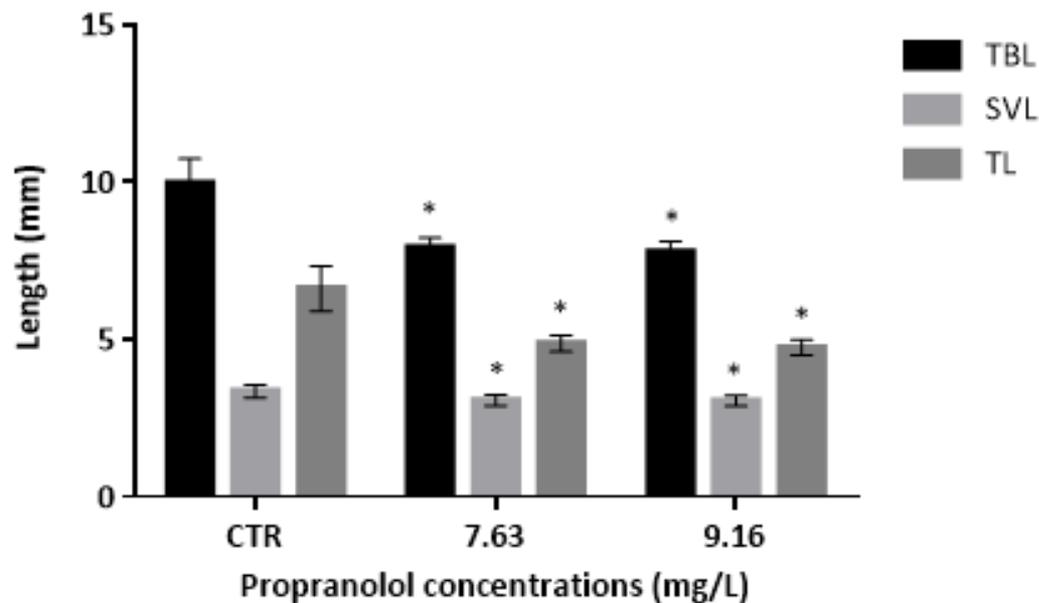
Average length of total body (TBL), snout-to-vent (SVL) and tail (TL) of *Xenopus laevis* larvae after for 96h exposure to propranolol concentrations. Error bars represent standard deviation. * indicates significant differences relatively to the respective control (p<0.001).

Results and Discussion

Propranolol – *P. perezi*

Tadpoles Survival

96h LC$_{50}$ - 3.58 mg/L
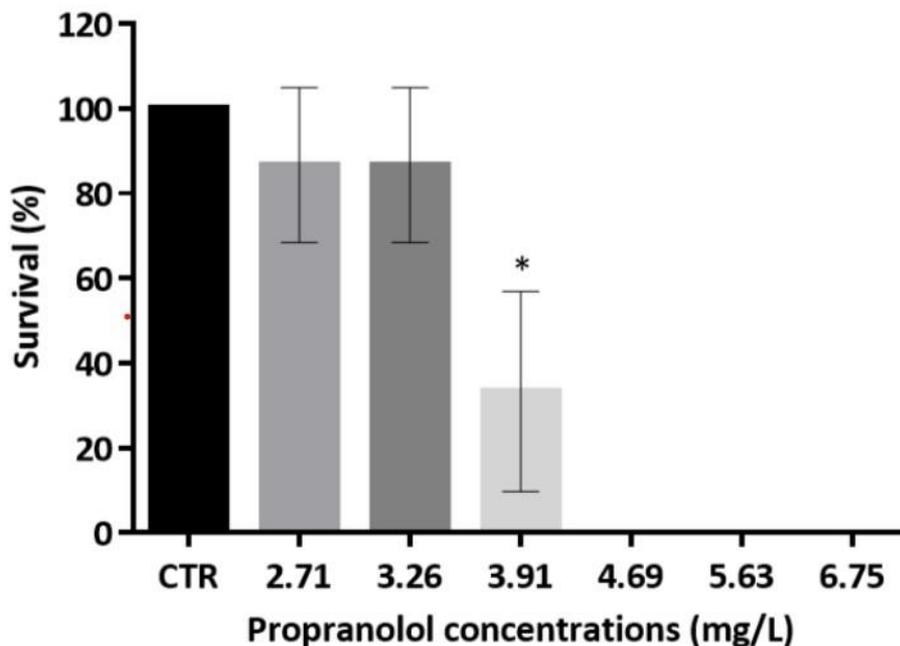
Survival of *Pelophylax perezi* tadpoles after 96h exposure to propranolol. Error bars represent standard deviation. * indicates significant differences relatively to the control ($p < 0.0001$).

Results and Discussion

Propranolol - *P. perezi*

    Tadpoles Malformations
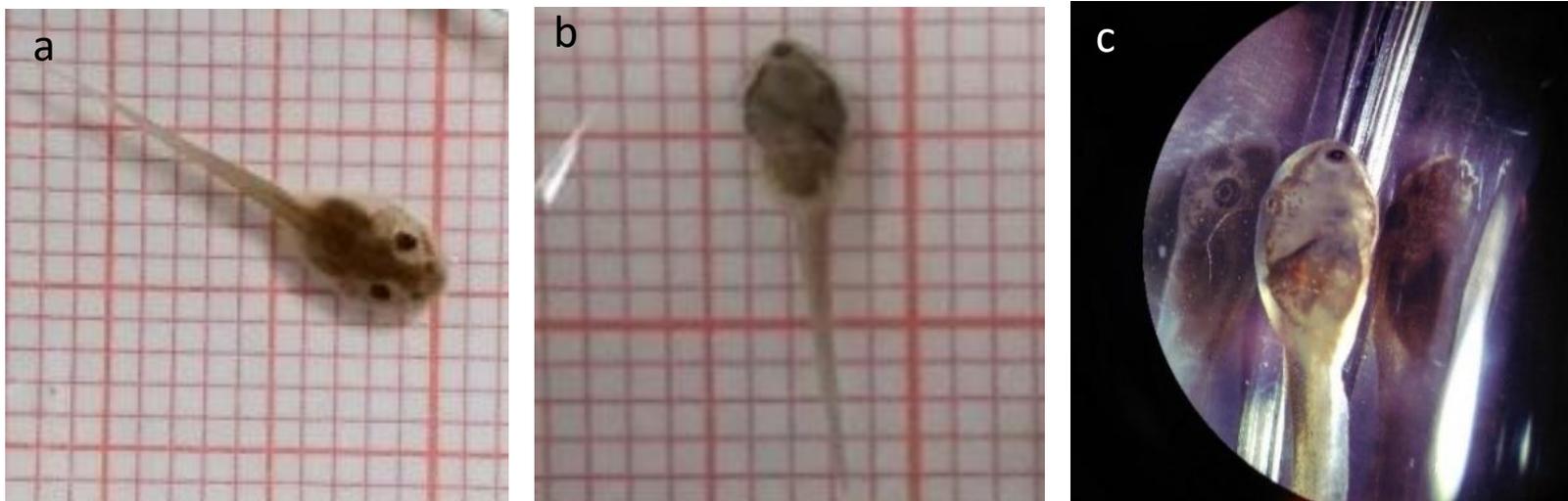
Examples of morphological effects *Pelophylax perezi* at the end of the tadpole toxicity assay.
(a)   Control tadpole;
(b)   Tadpole exposed to 2.71 mg/L propranolol without malformations;
(c)   Tadpole exposed 3.26 mg/L propranolol with a hemorrhage, the most common malformation in this assay.

Results and Discussion

Propranolol – *P. perezi*
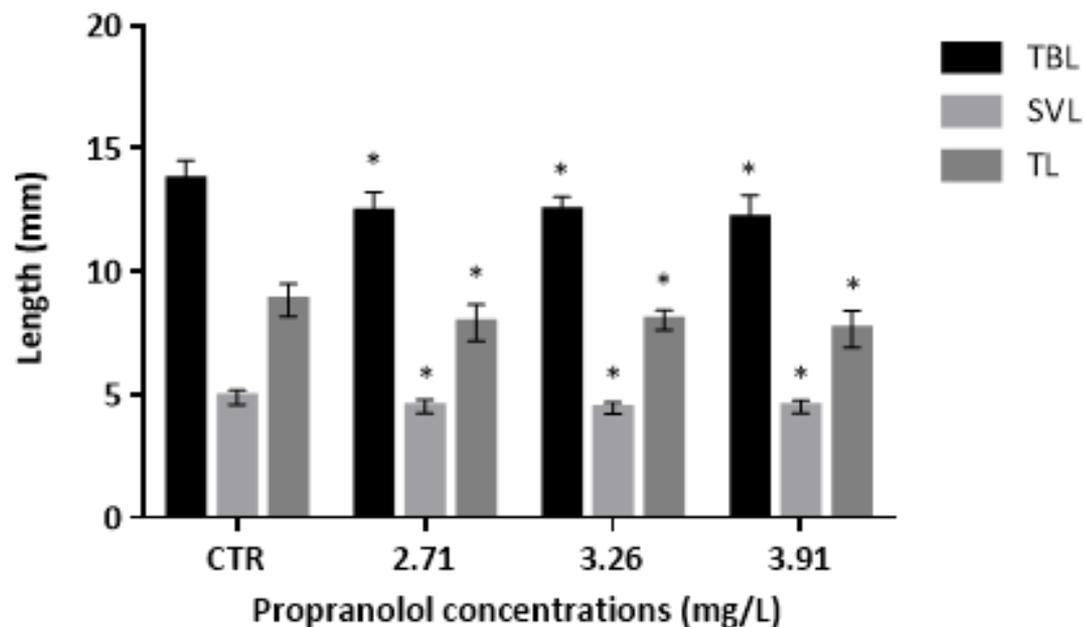
Tadpoles length

Average length of the tadpoles of *Pelophylax perezi*, after 96h exposure to propranolol. Error bars represent standard deviation. * indicates significant differences relatively to the control ($p < 0.001$). TBL-total body length; SVL-snout-to-vent length; TL-Tail length.

Results and Discussion

Propranolol – *In vitro effects*
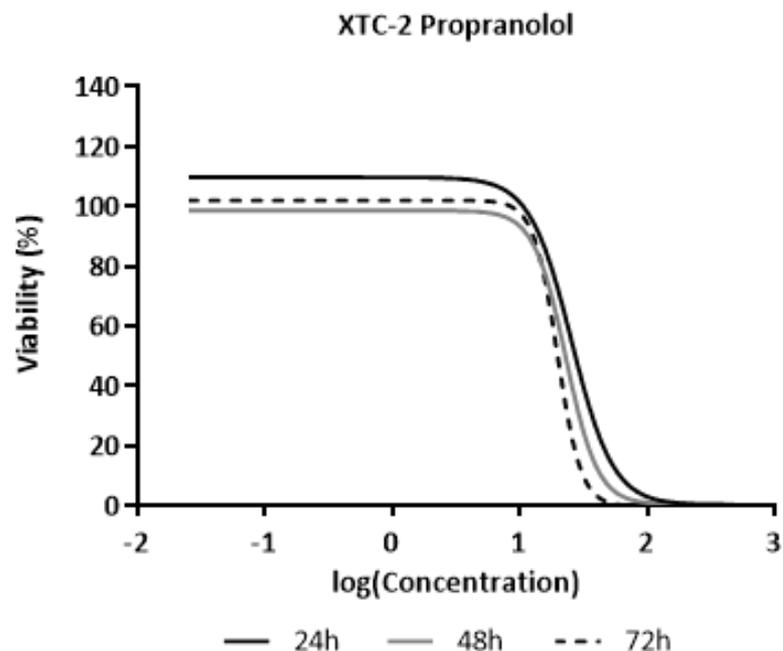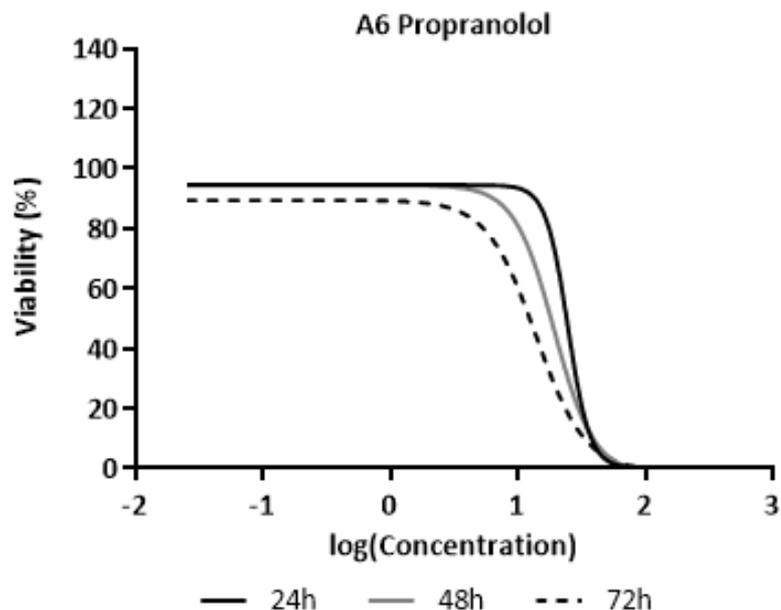
Cell viability

Cell viability of A6 and XTC-2 cells after exposure to propranolol at the three time points (24, 48 and 72h).

72h LC$_{50}$ – 12.4 mg/L   **72h LC$_{50}$ – 19.9 mg/L**

Results and Discussion

Haloperidol

Tadpoles Survival
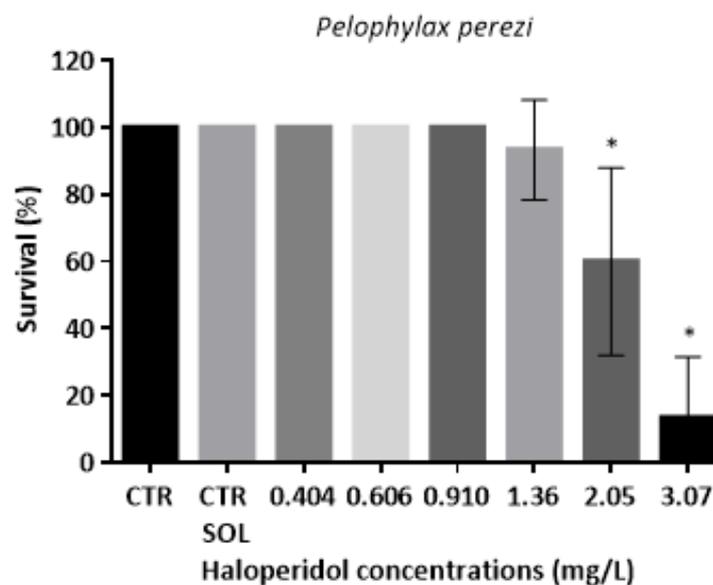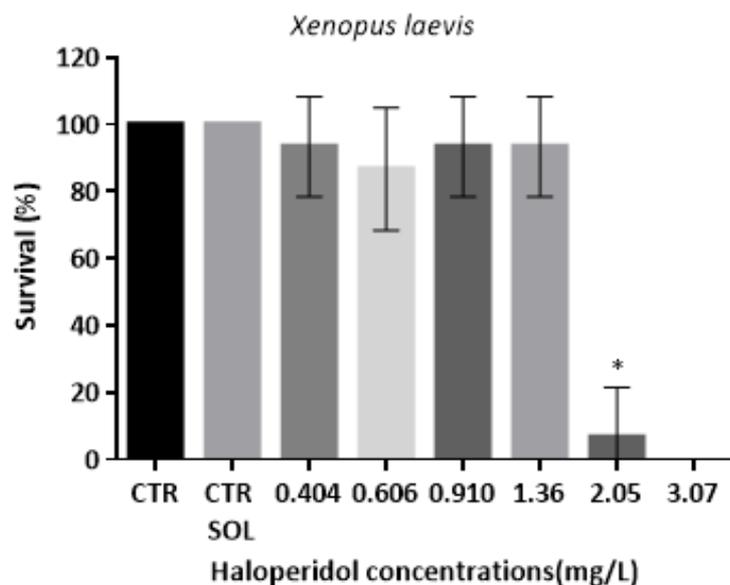
Average survival of *Xenopus laevis* and *Pelophylax perezi* tadpoles after 96h exposure to haloperidol. Error bars represent standard deviation. * indicates significant differences relatively to the control ($p < 0.05$) .

96h LC$_{50}$ - 1.45 mg/L   **96h LC$_{50}$ - 2.20 mg/L**

# Results and Discussion

Haloperidol

Tadpoles Weight
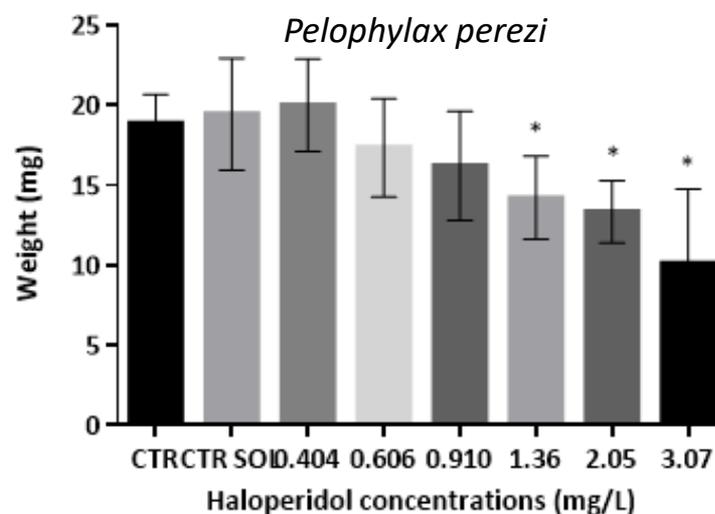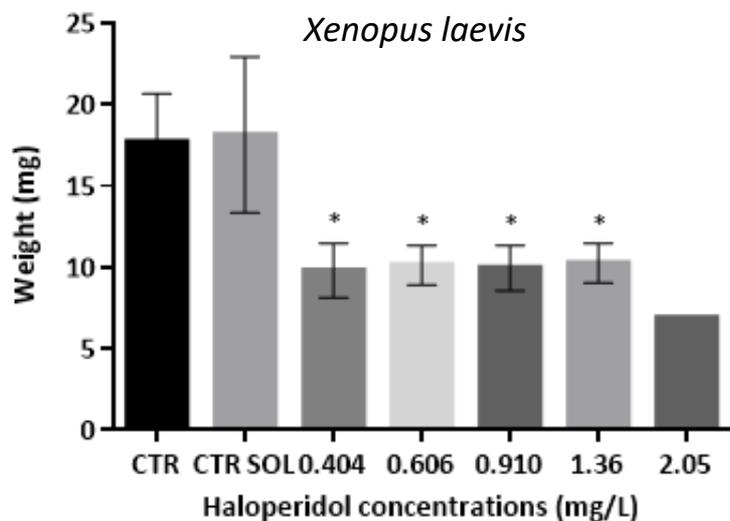
Average weight of tadpoles of *Xenopus laevis* and *Pelophylax perezi* after 96h exposure to haloperidol. Error bars represent standard deviation. * indicates significant differences relatively to the control ($p < 0.05$).

Results and Discussion

Haloperidol - *In vitro effects*
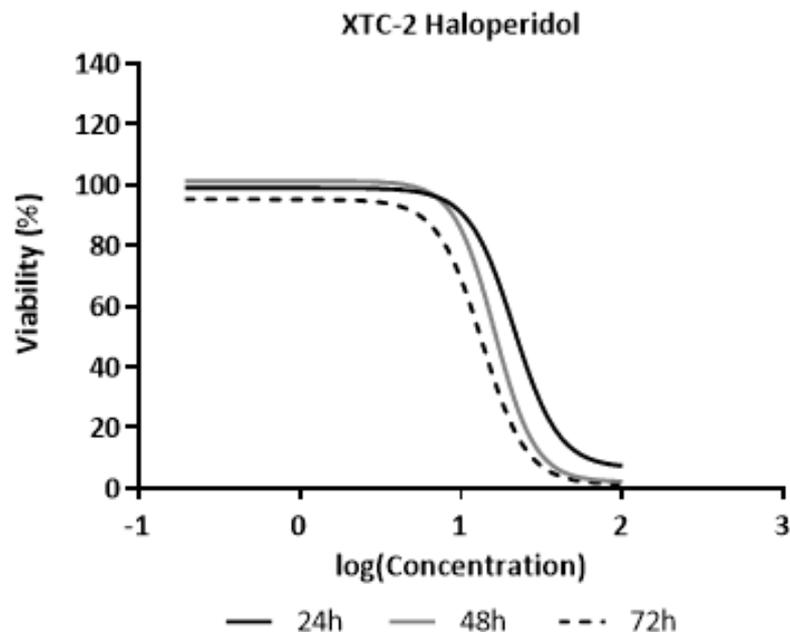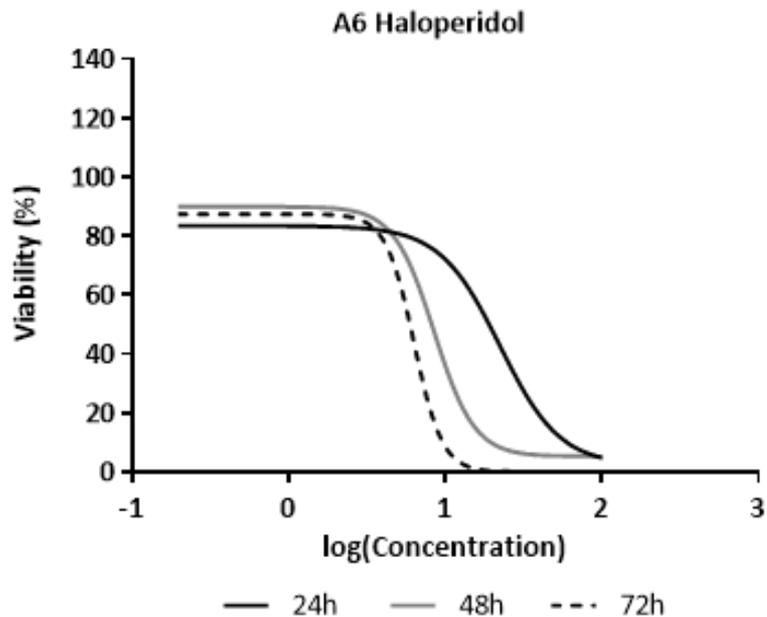
Cell viability

Cell viability of A6 and XTC-2 cells after exposure to haloperidol at the three time points (24, 48 and 72h).

72h LC$_{50}$ – 5.92 mg/L

72h LC$_{50}$ – 13.2 mg/L

Conclusions

The lethal concentrations in the *in vivo* assays were in the milligrams per liter range, considerably higher than the currently reported concentrations;

Data suggest low toxicity of these pharmaceuticals in acute exposures;

The *in vitro* approach demonstrated a lower sensitivity to pharmaceutical exposure than the early life stages of amphibians.

Acknowledgments

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