Ministério da Saúde

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Instituto Nacional de Controle de Qualidade em Saúde

INCQS

Assessing Amphotericin B polymeric nanoparticles' toxicity through Zebrafish FET test (OECD Test Guideline 236).

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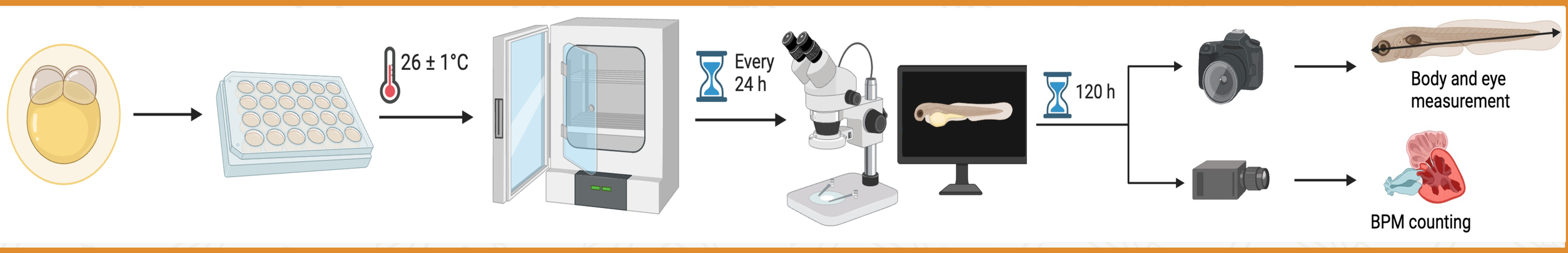
INTRODUCTION

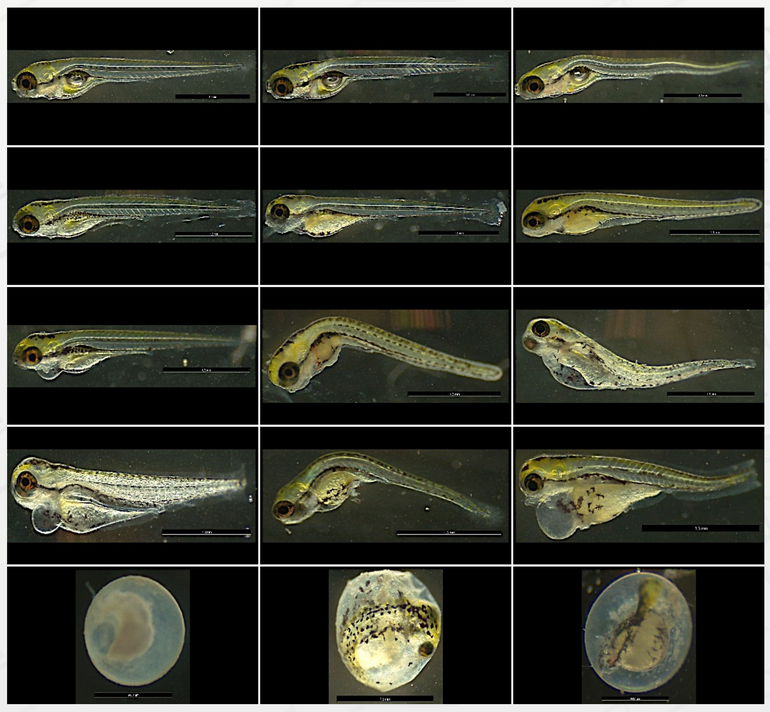
Amphotericin B (AmB) is a drug used mainly for the treatment of fungal infections. It is a low oral bioavailability drug, and presents an important nephrotoxicity related to dose and length of use. Polymeric nanoparticles (PNPs) are those consisting of synthetic or natural polymers, which have attracted the attention of the pharmaceutical industry due to characteristics such as the construction of controlled release models, improvement of bioavailability and reduction of adverse effects. The use of zebrafish (*Danio rerio*) as an animal model for toxicological studies has increased in recent years, and its fish embryo toxicity test (FET) has been widespread, due to the spped in obtaining a response and the possibility of estimating the LD_{50} for future tests in mammals. Since its first edition, in 2014, the ISO 16197 standard already presents the FET test as a relevant *in vivo* model for the study of toxicity of nanomaterials. Therefore, the goal of this work was to investigate the toxicity of polycaprolactone (PCL) and poly(lactic acid) (PLA) PNPs containing amphotericin B and not loaded correlates.

METHODOLOGY



RESULTS

All embryos exposed to AmB, at the concentrations of 4, 8, 16, 32 and 64 µg/mL, coagulated in less than 24 h. Therefore, all experiments were carried out only with Not Loaded (NL) PNPs, to determine if PLA and PCL are safe materials to test in a zebrafish model.



Construction of a semi-quantitative scale to classify the observed malformations:

0 = no malformations

1 = one malformation

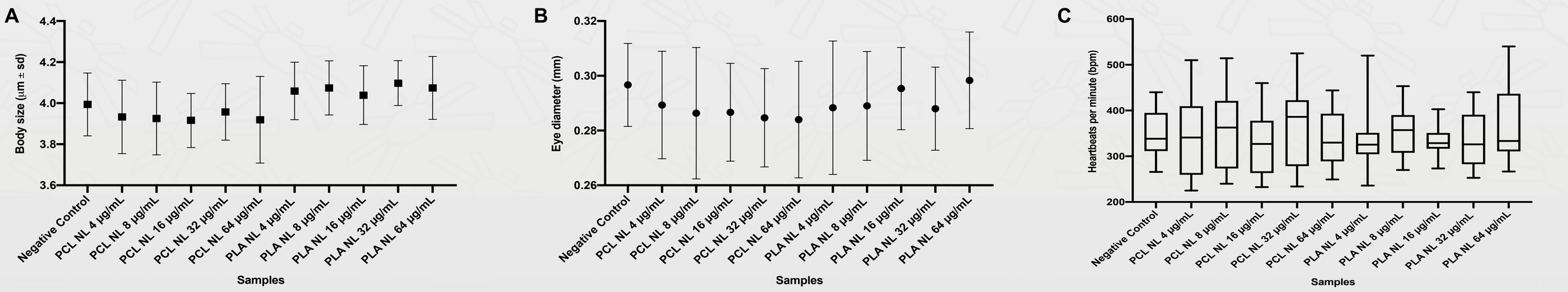
2 = two malformations

3 = three or more non-lethal alterations

4 = death or malformations that will cause death.

Groups	Points Frequency ± sd (%)				
	0	1	2	3	4
Negative Control[1]	81.7 ± 2.9	10.0 ± 5.0	-	-	8,3 ± 2,9
PLA NL 4 µg/mL	68.3 ± 7.6	5.0 ± 5.0	3.3 ± 5.8	3.3 ± 5.8	20,0 ± 0,0
PLA NL 8 µg/mL	75.0 ± 5.0	10.0 ± 5.0	-	3.3 ± 5.8	11,7 ± 5,8
PLA NL 16 µg/mL	71.7 ± 7.6	10.0 ± 5.0	5.0 ± 8.6	-	13,3 ± 2,9
PLA NL 32 µg/mL	75.0 ± 10.0	13.3 ± 7.6	-	1.7 ± 2.9	10,0 ± 5,0
PLA NL 64 µg/mL	81.7 ± 15.3	5.0 ± 8.7	1.7 ± 2.9	-	11,7 ± 7,6
PCL NL 4 µg/mL	75.0 ± 10.0	13.3 ± 7.6	5.0 ± 5.0	-	6,7 ± 7,6
PCL NL 8 µg/mL	66.7 ± 11.6	10.0 ± 10.0	3.3 ± 5.8	3.3 ± 2.9	16,7 ± 7,6
PCL NL 16 µg/mL	70.0 ± 13.2	21.7 ± 15.3	-	1.7 ± 2.9	6,7 ± 2,9
PCL NL 32 µg/mL	76.7 ± 5.8	6.7 ± 7.6	3.3 ± 2.9	3.3 ± 2.9	10,0 ± 5,0
PCL NL 64 µg/mL	66.7 ± 15.3	10.0 ± 5.0	5.0 ± 5.0	-	18,3 ± 14,4

[1]E3 medium. PLA, poli(D,L-lactic acid); PCL, policaprolactone; NL, not loaded; sd, standard deviation. Results shown as percentage average ± sd of three independent replicates, with n = 20 embryos each.



A: Body size; B: Eye diameter; C: Heartbeats per minute. Images, videos and measurements taken at 120 hours post-fertilization of zebrafish larvae, using Leica S9i stereoscope and LASX software. No differences were observed between the negative control group and the groups exposed to not loaded polymeric nanoparticles at same dilutions applied to prepare the AmB concentrations of 4, 8, 16, 32 and 64 µg/mL.

CONCLUSION

Regarding AmB toxicity, it is possible to conclude that, at the studied concentrations, the drug is lethal for zebrafish embryos, even when nanoformulated in PCL or PLA PNPs. However, concerning the safety of PLA and PCL, it can be concluded that they proved to be safe in the zebrafish embryo test, since they did not present statistical differences in relation to the control group in any of the concentrations tested and in any of the evaluated parameters.

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