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ABSTRACT

Zebrafish (Danio rerio) is commonly used as test organism in developmental biology, genetics and in ecotoxicology. Since last few years, it has attracted researchers because of the higher sensitivity of embryos and larvae, compared to adult fish. In addition, it is a non-mammalian organism that can bridge gaps between in vitro cell systems and in vivo studies. The present study was performed to determine the toxic effects of 3,4-Dichloroaniline (DCA) in zebra fish embryo and sac-fry stages (OECD 212). DCA has been widely used for the synthesis of several herbicides, azo dyes for textiles and pharmaceuticals. DCA has potential to damage fish embryos. The study was performed under semi-static condition where media was renewed at 24 h intervals. Fertilized 1.2 hour-post-fertilization (hpf) zebrafish embryos were exposed at concentrations of DCA ranging from 0.09 to 3.0 mg/L in 24 well culture plates along with controls, and incubated at 26 ± 1 °C for 11 days. Dose-dependent biologically significant increase in morphological changes and mortalities were observed at the concentrations of 0.19 - 3.0 mg DCA/L compared to control. The morphological changes observed in test groups were coagulation of embryos, non-detachment of tail, lower frequency of heartbeat, curved body and pericardial oedema as compared to control. The no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) over the 10 days exposure period of DCA were 0.09 and 0.19 mg/L, respectively. The 96-hour EC_{50} value for hatching of embryo was 2.83 mg/L and 10-day LC_{50} value was 1.11 mg/L. These results support the use of zebrafish embryos to evaluate water pollutants.

TOXICITY OF 3,4-DICHLOROANALINE ON ZEBRAFISH EMBRYO AND SAC-FRY STAGES

INTRODUCTION

Zebrafish is commonly used as test organism in developmental biology, genetics and in ecotoxicology. Since last few years, it has attracted researchers because of the higher sensitivity of embryos and larvae, compared to adult fish. In addition, it is a non-mammalian organism that can bridge gaps between in *vitro* cell systems and *in vivo* studies. 3,4-dichloroanlaine has potential to damage fish embryos. The present study was performed to determine the toxic effects of 3,4-dichloroanaline (DCA) on zebrafish embryos and sac-fry stages.

OBJECTIVE

Present study was conducted to elucidate the effect of 3,4dichloroanaline on zebrafish (Danio rerio) embryo and sac-fry stages.

MATERIAL METHOD

Test Item

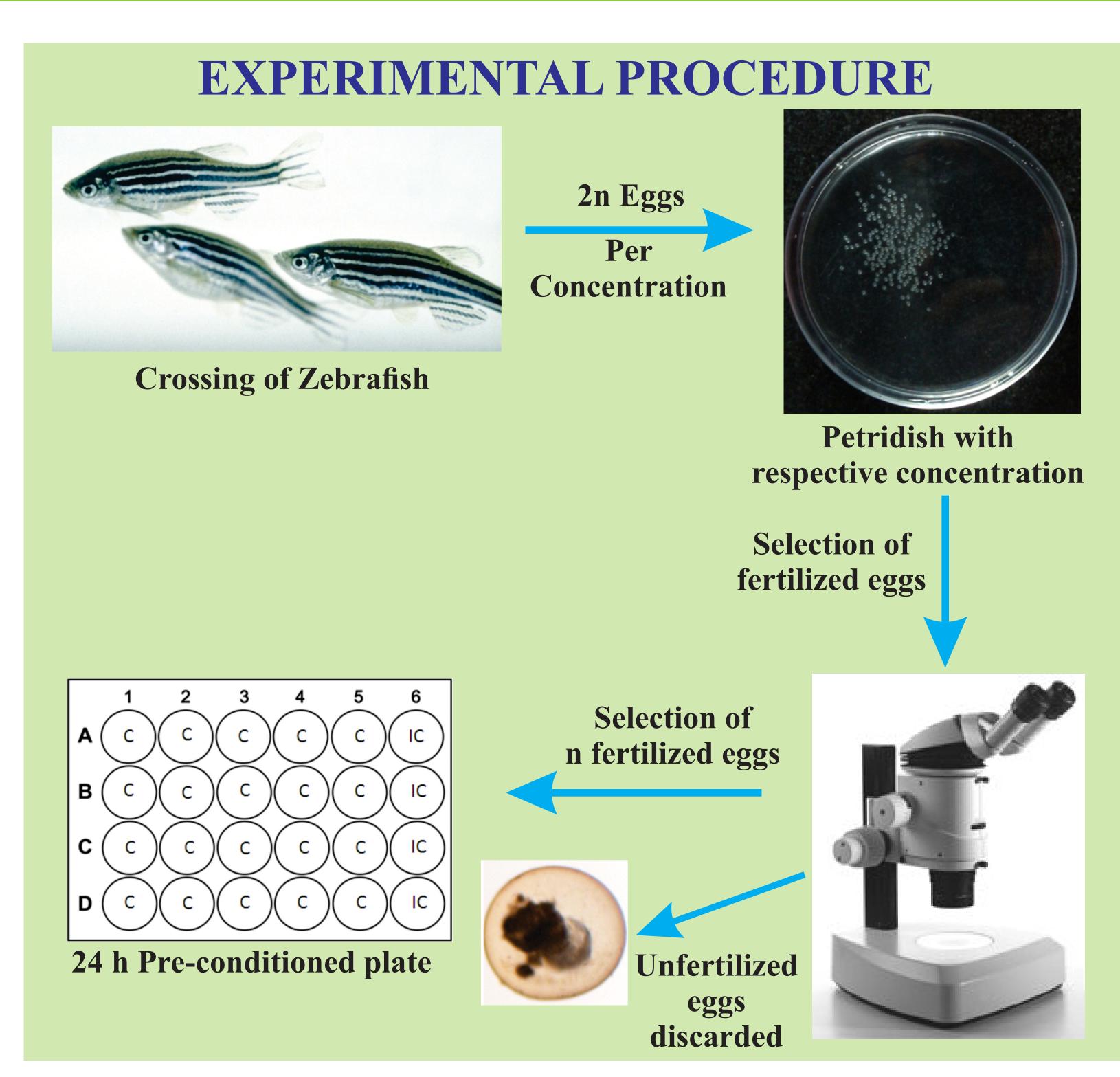
3,4-dichloroaniline (97.6%), Sigma-Aldrich, Switzerland.

Fish

Adult wild type zebrafish (*Danio rerio*) were used in the study. They were housed in Zebrafish Hosing System (Aquaneering, USA).

Test System	Zebrafish Embryos
Test Media	E3 Medium
Test Procedure	Semi-static
Control	Test medium (E3 Medium)
Number of Embryos	30 embryos per concentration (10 embryos/replicate)
Test Duration	Till mortality observe in control due to starvation
Observation	Every 24 h interval
Test Concentrations	0.0 (Control), 0.09, 0.19, 0.38, 0.75, 1.5 and 3.0 mg/L

EXPERIMENTAL DESIGN



RESULT

The no observed effect concentration and lowest observed effect concentration over the 10 days exposure period of 3,4dichloroaniline were 0.09 and 0.19 mg/L, respectively. The 96 h, EC₅₀ value for hatching of embryos was 2.83 mg/L and $10 \text{ day}, LC_{50} \text{ value was } 1.11 \text{ mg/L}.$

CONCLUSION

Based on the result of the present study, it can be concluded 3,4-dichloroaniline shows toxic effect on zebrafish embryo and sac-fry stages.

REFERENCES

BIS, 1983: Methods of Sampling and Test (physical and chemical) for "Water and Wastewater", Part 21Total Hardness, First Revision. IS : 3025 (Part 21), Bureau of Indian Standard, adopted December 30, 1983.

Finney D.J., 1971: Probit Analysis. 3rd Edition, Cambridge, the University Press, p. 333.



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- OECD, 1998: OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1, "OECD Principles on Good Laboratory Practice" ENV/MC/CHEM(98)17 (as revised in 1997).
- OECD, 1998: OECD N° 212, Fish, Short-term Toxicity Test on Embryo and Sac-fry Stages", Organisation for Economic Co-operation and Development (OECD) Guidelines for Testing of Chemicals, adopted by the Council on September 21, 1998.
- http://cshprotocols.cshlp.org/content/2011/10/pdb.rec66449.full?text_on ly=true.
- EC, 2008: C.15, "Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages, The Official Journal of the European Economic Community (L 142): Part C: Methods for the Determination of Ecotoxicity," Council Regulation (EC) No. 440/2008 of 30 May 2008.

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