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The Embryotoxicity of Some Phenol Derivatives on Zebrafish, *Danio rerio*

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ABSTRACT

The existence of toxicants in ecosystems has been increased dramatically in recent years, especially in aquatic environments. Phenols and chlorinated phenol derivatives are toxic industrial compounds. Phenols and derivatives are known to be environmental contaminants. In the present study, 2,4-Dichlorophenol, 2-Chlorophenol and substituted phenol were tested for embryotoxicity and mortality in a four-day period using zebrafish, *Danio rerio* embryos. Tested phenol derivatives caused teratogenicity and embryo mortality in the embryos. The semi static 48-h LC_{50} (median lethal concentration) value for Substituted-phenol was 13.850 mg L^{-1}; the corresponding values for 2-Chlorophenol and 2,4-Dichlorophenol were 8.378 mg L^{-1} and 6.558 mg L^{-1}, respectively. The endpoints are incomplete eyes, head and tail, heart and chorda deformity, yolk sac edema, tail curvature, shrunken eyes, lordosis, delayed hatching, weak pigmentation, heart edema and non-pigmentation after exposure to the compounds. 2,4-Dichlorophenol was found to be more toxic than the others. This paper is the first to describe the relative toxicity of a suite of phenols in the early life stages of zebrafish.

Key words: Substituted phenol, 2-Chlorophenol, 2,4-Dichlorophenol, zebrafish, embryotoxicity.

INTRODUCTION

An increasing diversity of environmental pollutants are entering the aquatic ecosystem and causing potential long-term negative effects on organisms in aquatic system (Livingstone 1998; Livingstone 2001). Phenols and chlorinated derivatives are industrial toxic compounds (Pera-Titus *et al.* 2004). These pollutants originate from different sources such as the effluents of the petrochemical industries, and various chemical manufacturing industries (herbicides, pesticides, solvents, paints, plastics, etc.) (Arana *et al.* 2001). Phenols are widely used in the synthetic chemical industry, dying, mining and agriculture, and they are organic pollutants. Phenols and their chlorinated derivatives are resistant to biodegradation processes. Therefore, phenols may accumulate in aquatic biota and negatively affect all aquatic organisms (Czaplicka 2004). Chlorophenols (CPs) are extensively used as by-product of bleaching in paper mills, as wood treatment agent and for biocide production. CPs also show a very wide distribution in the environment (Stringer & Johnston 2001). Di-, tri- and penta-chlorophenol are classified as priority pollutants by USEPA since CPs have adverse effects on human and wildlife, such as chronic toxicity, mutagenicity and carcinogenicity (Ramamoorthy & Ramamoorthy 1997). 2-Chlorophenol (2CP) is on the priority pollutant list of the USEPA and is used in pulp, paper and pesticide industries (Dec *et al.* 2003). The USEPA has also recommended restricting 2CP and 2,4-Dichlorophenol (2,4-DCP) concentrations in freshwaters to below 4380 and 2020-ug L^{-1}, respectively (USEPA 1980a; USEPA 1980b). It is considered that industrial waste discharge including chlorophenols creates major water pollution (Krijgsheld & Van der Gen 1986). Some phenols involved in surface waters are 2-CP, 2,4-DCP and 2,4,6-TCP (Scow *et al.* 1982). The most abundant phenol in aquatic environments is 2,4-DCP (House *et al.* 1997). 2,4-DCP was determined in drinking water supplies in the USA and the highest detected concentration was 36-ug L^{-1} (Shackelford & Keith 1976). The toxicity of phenol and derivatives were widely studied on some invertebrates and vertebrates. For example, Prati *et al.* (2000) and Qiao *et al.* (2006) reported that phenols induced genotoxic effects in animals and human. Although previous studies were focused on the general toxicity of phenols, few studies investigated the developmental toxicity of the

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compounds. Therefore, the aim of this work was to evaluate the developmental toxicity of phenol derivatives by means of zebrafish bioassay.

Zebrafish, *Danio rerio* (Hamilton, 1822) is a valuable test organism, especially for toxicity research. The fish has transparent embryos and short spawning time (Nagel 2002). In addition, zebrafish is a model organism, and has many advantages such as being easy to maintain and breed, inexpensive and providing rapid assay for acute and chronic toxicity (Westerfield 2007). The objectives of the research were i) to determine the median lethal concentrations (LC_{50}) of substituted phenol, 2-Chlorophenol and 2,4-Dichlorophenol for 48 hpf embryos of zebrafish, ii) to study the teratogenic effects of the phenol derivatives on the development of the fish, and iii) to compare the toxicity of the three phenol compounds.

MATERIALS AND METHODS

Chemicals

Substituted phenol (SP), 2-Chlorophenol (2-CP) and 2,4-Dichlorophenol (2,4-DCP), were obtained from Sigma-Aldrich (Darmstadt-Germany). Before starting the test, stock solutions of the phenol derivatives were prepared by dissolution in low-conductivity water prepared from a MiliQ water treatment system, and stock solutions were stored at 5 °C.

Fish culture and egg production

Adult zebrafish samples were provided by Atatürk University Fisheries Faculty Research Centres for Aquarium Fish. Before the experiments, the fish were acclimatised for 14 days. Twenty healthy adult fish were placed in each aquarium with a photoperiod 14:10 light and dark cycle. Dechlorinated municipal water was used in aquaria and maintained at 27±1 ºC. The water was renewed at 1/3 ratio every week.

The fish were fed with dry flake food twice a day, and live feed was fed once every two days. Breeding groups were formed and placed separately in small spawning aquarium which were equipped with glass balls on the bottom. No air filters were used in the spawning aquaria and fish were not fed during breeding. When the light was turned on, spawning was induced in the morning. Half an hour later, healthy and fertilized eggs were collected and placed in fresh embryo medium (Hank's solution), and incubated at 27±1 ºC until treatment.

Embryotoxicity and Teratogenicity

The fish embryos were exposed to phenol concentrations to determine the 50% lethal concentrations (LC_{50}). The experimental concentrations of SP, 2CP and 2,4-DCP (2, 4, 8, 16 and 32 mg L^{-1}) were inspired by previous work (Nagel 2002). Hank's solution was used as control medium. At approximately 3 hour post-fertilization (hpf), blastula stage embryos were selected under a stereomicroscope in Hank's solution. The exposure was begun after placing the fertilised eggs into the test solutions. Twenty embryos were used in each group including control. Each experiment was repeated three times (total 360 embryos for one phenol compound). Phenol exposure to embryos was performed in a glass petri dish (10 cm diameter) containing 50 ml test solution at 27±1 ºC with photoperiod of 14:10-h light/dark cycle in a precision incubator until 96 hpf.

Test solutions were renewed with fresh solutions every 24-h (semi-static test condition). Every 12-h, the embryos were observed and scored for lethal and sublethal effects using a microscope with a digital camera. Embryo-larva stages were categorized as described by Kimmel *et al.* (1995). According to Lammer *et al.* (2009), lethal and sublethal effects were determined. Lethal abnormalities (coagulation, missing heartbeat, somites, tail detachment and spontaneous movement) were determined for each group. Then, dead embryos or larvae were immediately removed and recorded at each observation time. Some teratogenic malformations (incomplete eyes, head and tail, heart and chorda deformity, yolk sac edema, tail curvature, lordosis, heart edema, non-pigmentation) were also recorded. The embryos and larvae were scored for malformations at 72-hpf according to the rating scale by Padilla *et al.* (2011).

Each embryo or larva was marked for various categories such as curved spine, non-hatching and edema on the scale. Each of the categories included a number of abnormalities scored as yes/no or as degree. Total Malformation Index (MI) was calculated using the scores from each category for each embryo. MI values of 0-3 MI indicated as normal; 4-6 values as slightly abnormal, and values above 7 as obviously deformed embryo or larva.

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Statistical analysis

SPSS (version 20.0) software programme was used for statistical analysis. The 48-h LC_{50} values were determined by probit analysis. Statistical differences in the rate of embryo lethality and number of abnormal embryos/larvae were evaluated with One-Way ANOVA. All data were expressed as mean ± standard deviation (SD). For multiple comparisons, analysis of variance was used followed by Dunnett's test. $p<0.05$ was accepted as the statistical significance level.

RESULTS

Calculated 48-h LC_{50} values by probit 95% confidence interval (CI) for SP, 2CP and 2,4-DCP were 13.850, 8.378 mg L^{-1} and 6.558 mg L^{-1} for zebrafish embryos, respectively. According to the values, 2,4-DCP was more toxic than 2CP and SP. LC_{50} values of the phenolic compounds for zebrafish embryos were firstly reported in this study (Figs. 1, 2 and 3).

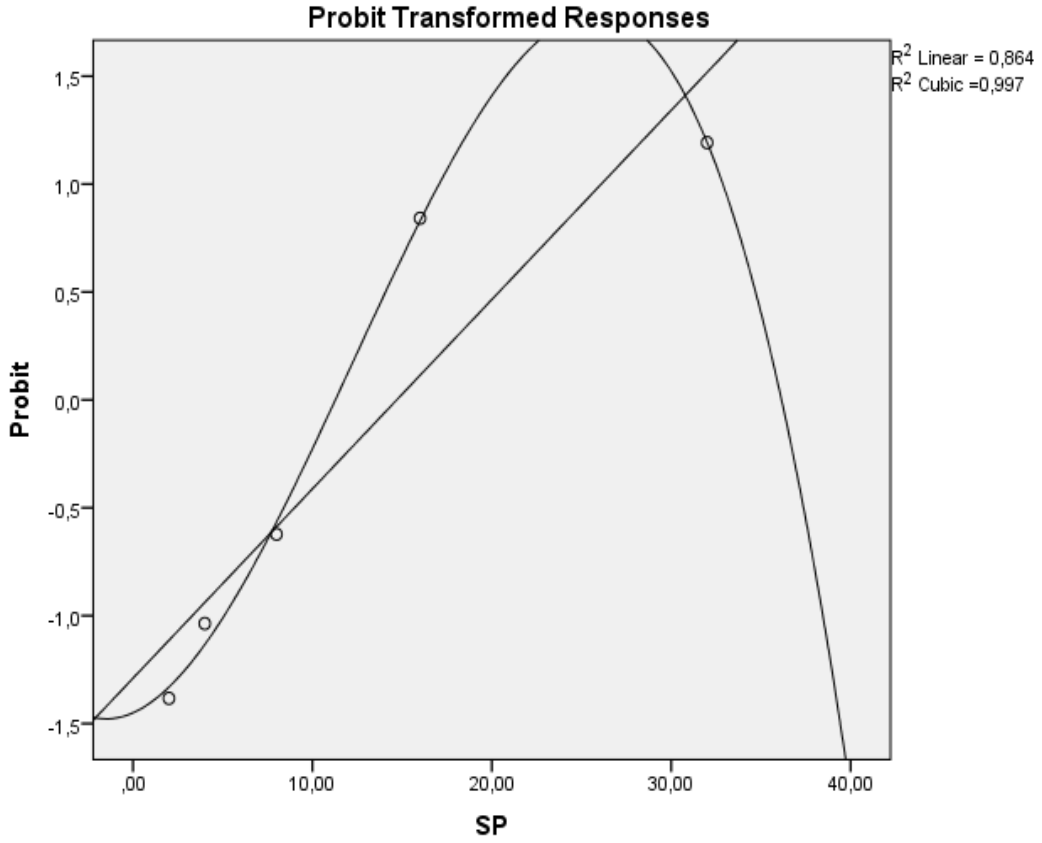

Fig. 1. Dose-response curves used to calculate the LC value. LC curve of SP (95% confidence limits).

The developmental rate was slow in experimental groups comparing to control group. Table 1 shows that some developmental parameters such as gastrulation and somite completion, optic cup formations, spontaneous contraction, tail detachment, heartbeat, blood circulation, pigmentation of and hatching in zebrafish embryos occurred at the slowest rate in living embryos exposed to sublethal concentrations of SP, 2CP and 2,4-DCP. It was found that phenol derivatives reduced the number of hatched embryos. Also, the larvae were found to stay within the chorion and never hatched with high phenol concentrations at the end of exposure. Zebrafish embryos showed over 50% mortality in 48-h at high concentrations of the phenols. Mortality was not found to be significantly different from each other with low concentrations of the phenols. The frequency of dead embryos was distributed throughout the 48-h exposure period. Lethal and teratogenic effects were recorded at 96-hpf (Tables 2, 3 and 4). Especially, it was observed that the percentage of affected and dead embryos increased with high phenol concentrations. Table 2 shows that 16 and 32 mg L^{-1} SP caused embryo mortality within two days, reaching 80% mortality at 16 mg L^{-1}.

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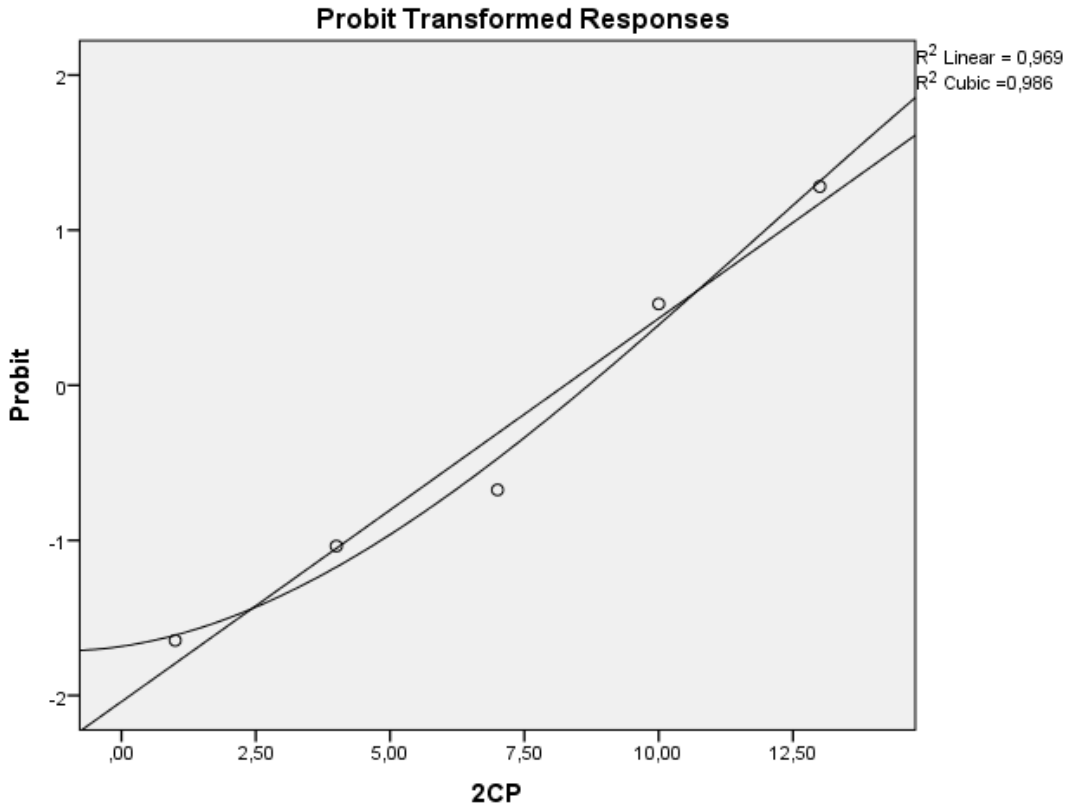

Fig. 2. Dose-response curves used to calculate the LC value. LC curve of 2CP (95% confidence limits).

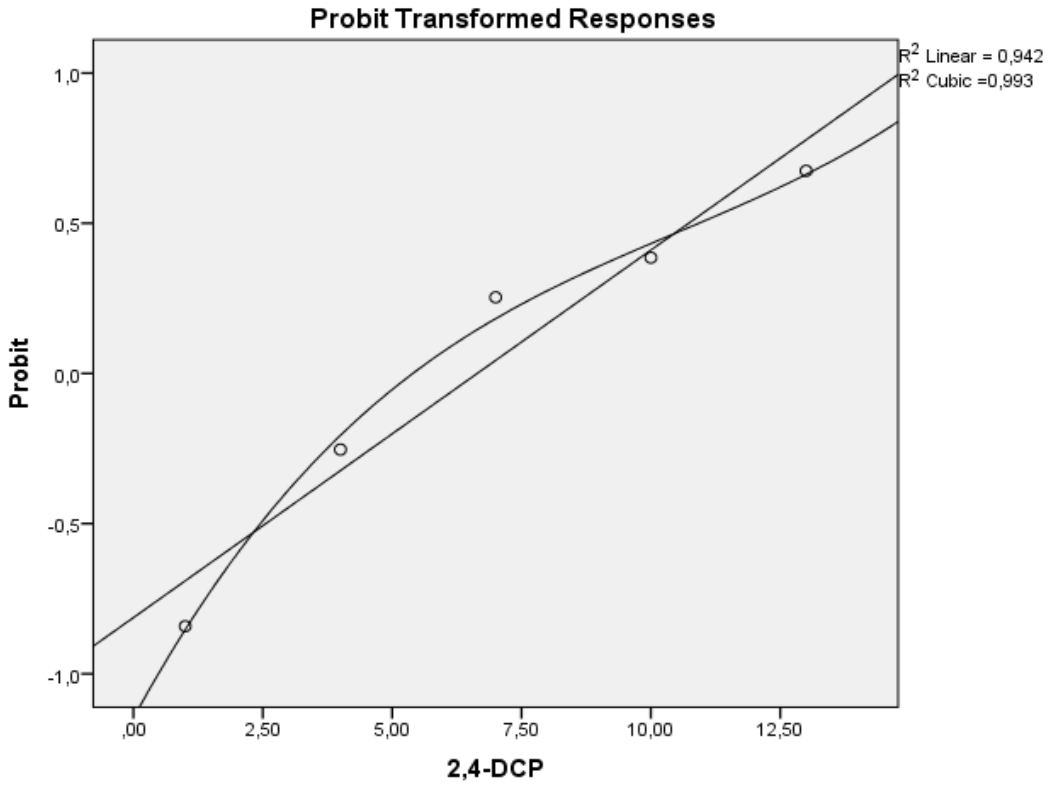

Fig. 3. Dose-response curves used to calculate the LC value. LC curve of 2,4-DCP (95% confidence limits).

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Table 1. The Rates of development in zebrafish embryos.

Developmental period[a] Age	Cleavage 1.5 hpf	75 % epiboly 8 hpf	Pharyngula 24 hpf	Hatching 48 hpf	Early larva 80 hpf
Control	1.6 ± 0.20	8.6 ± 1.13	25.1 ± 2.21	49.0 ± 3.06	81.0 ± 3.29
SP (8 mg L^{-1})	1.7 ± 0.15	12.0 ± 0.42*	32.6 ± 3.45*	57.5 ± 4.80*	108.5 ± 5.89*
2CP (4 mg L^{-1})	1.7 ± 0.10	16.1 ± 0.76*	37.5 ± 4.86*	66.4 ± 5.13*	110.3 ± 8.56*
2,4DCP (4 mg L^{-1})	1.6 ± 0.10	18.9 ± 0.91*	46.6 ± 4.60*	79.2 ± 7.34*	131.1 ± 9.58*

[a] Described by Kimmel *et al.* (1995).
The values are presented as mean ±SD. "hpf" shows hour of development periods. *Significantly different from control at $p < 0.05$

Table 2. Adverse effects of SP in the embryos at 96-hpf.

	Control	2 mg L^{-1}	4 mg L^{-1}	8 mg L^{-1}	16 mg L^{-1}	32 mg L^{-1}
Number of teratogenic embryos	3	3	6	20	5	0
Number of dead embryos	3	6	9	26	43	60
Number of affected embryos	6	9	15	46	48	60
Number of normal embryos	54	51	45	14	12	0
% Teratogenic embryos	5.0 ± 1.2	5.0 ± 1.5	10 ± 1.0	33.3 ± 2.9	8.3 ± 1.6	0
% Dead embryos	5.0 ± 1.1	10 ± 1.7	15 ± 2.6	43.3 ± 4.6	71.7 ± 6.5	100 ± 0
% Affected embryos	10 ± 1.0	15 ± 2.5	25 ± 2.7	76.7 ± 6.7*	80 ± 6.1*	100 ± 0*
% Normal embryos	90 ± 5.8	85 ± 5.2	75 ± 6.6	20 ± 2.4	20 ± 4.1	0

* Significantly different from control at $p < 0.05$.

At two highest concentrations of 2CP, many of the early life stage abnormalities were persistent and resulted in embryo mortality in the next days, reaching 90% (Table 3). All embryos showed teratogenicity in 2CP group coagulated at the end of 96 hpf. A dose-response relationship was detected in the percentage of embryos with lethal and teratogenic effects after exposure to 2,4-DCP (Table 4). At the 4 and 8 mg L^{-1}, teratogenic effects were observed at 96-hpf, whereas at 16 and 32 mg L^{-1} these effects were found at 48-hpf. During the early life stages of development (< 48-h), observed abnormalities included incomplete eyes, head and tail development (Fig. 4B), heart and chorda deformity (Fig. 4D*).*

After hatching, clear macroscopic aberrations included yolk sac edema, tail curvature and shrunken eyes (Fig. 5B), lordosis (Fig. 5C), delayed hatching and weak pigmentation (Fig. 5D), heart edema, tail curvature and depigmentation (Fig. 5E) observed in all treated embryos. No incidence of malformations was observed in the controls (Fig. 4A and 4C, Fig. 5A). The most dominant abnormalities were detected as chorda malformations. All of teratogenic embryos and larvae died within 1-2 days after 96-hpf.

Table 3. Adverse effects of 2-CP in the embryos at 96-hpf.

	Control	2 mg L^{-1}	4 mg L^{-1}	8 mg L^{-1}	16 mg L^{-1}	32 mg L^{-1}
Number of teratogenic embryos	2	5	16	19	8	0
Number of dead embryos	2	4	7	24	47	60
Number of affected embryos	4	9	23	43	55	60
Number of normal embryos	56	51	37	17	5	0
% Teratogenic embryos	3.3 ± 0.2	8.2 ± 1.5	26.6 ± 2.0	31.6 ± 1.9	13.3 ± 1.6	0
% Dead embryos	3.4 ± 0.3	6.6 ± 0.3	11.6 ± 1.6	40.6 ± 3.6	78.3 ± 4.9	100
% Affected embryos	6.7 ± 0.5	15.2 ± 3.5	38.3 ± 2.2*	71.6 ± 4.0*	91.6 ± 5.3*	100*
% Normal embryos	93.3 ± 6.2	85.3 ± 5.2	61.6 ± 4.6	28.3 ± 2.1	8.3 ± 1.4	0

* Significantly different from control at $p < 0.05$.

Table 5 shows that mean total Malformation Index (MI) for 2 mg L^{-1} phenol congeners were not statistically different from the control groups. However, the mean total MI increased in 72-hpf embryos at 4, 8 and 16 mg L^{-1} phenol concentrations. The embryos in control and 2 mg L^{-1} phenols were normal because they were scored between 0 and 3 for MI. The other embryos were scored between 4 and 6, and were slightly abnormal (in 4 and 8

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mg L^{-1} phenols); and in the 16 mg L^{-1} phenols, embryos scored above 7 were absolutely deformed. MI could not be calculated for embryos in 32 mg L^{-1} because all of them died in 72-hpf.

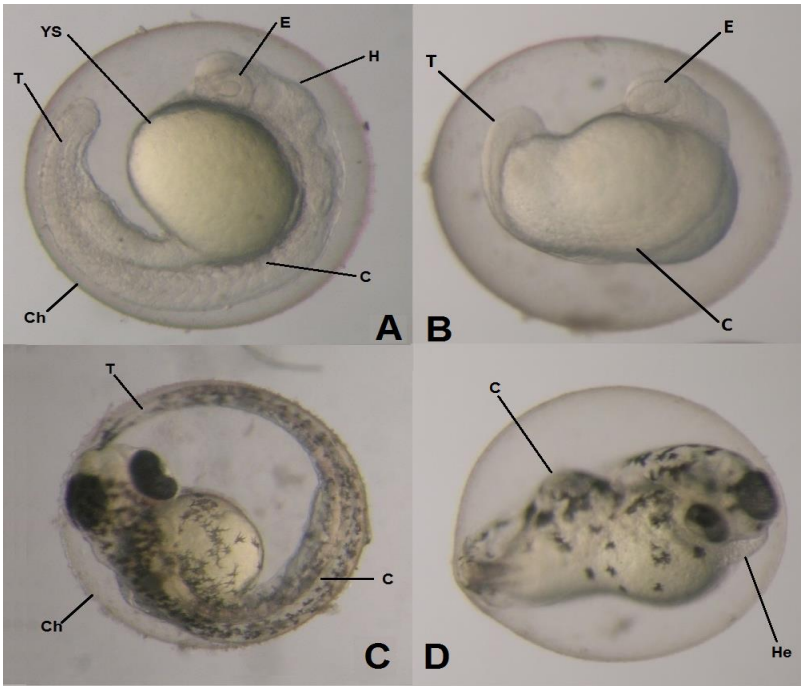

Fig 4. Photomicrograph of malformations in zebrafish embryos exposed to 16 mg L^{-1} SP, 8 mg L^{-1} 2CP and 4 mg L^{-1} 2,4-DCP. A) 24-hpf normal embryo showing somites, eyes, head and chorda B) 24-hpf abnormal embryo showing incomplete eyes, head and tail development C) 48-hpf normal embryo showing pigmentation and normal chorda. D) 48-hpf abnormal embryo showing heart and chorda deformations (48-hpf). C: chorda, Ch: chorion, E: eyes, H: head, He: heart, T: tail, YS: yolk sac.

Table 4. Adverse effects of 2,4-DCP in the embryos at 96-hpf.

	Control	**2 mg L^{-1}**	**4 mg L^{-1}**	**8 mg L^{-1}**	**16 mg L^{-1}**	**32 mg L^{-1}**
Number of teratogenic embryos	2	5	18	11	5	0
Number of dead embryos	1	5	11	34	50	60
Number of affected embryos	3	10	29	45	55	60
Number of normal embryos	57	50	31	15	5	0
% Teratogenic embryos	3.3 ± 0.5	8.3 ± 1.5	30.0 ± 2.0	18.5 ± 2.9	8.3 ± 2.9	0
% Dead embryos	1.6 ± 0.1	8.2 ± 2.5	18.3 ± 2.6	56.6 ± 5.6	83.3 ± 7.2	100
% Affected embryos	5.3 ± 0.6	16.6 ± 1.8*	48.3 ± 4*	75.6 ± 5*	91.6 ± 7.9*	100*
% Normal embryos	95.6 ± 6.9	83.3 ± 4.0	51.6 ± 5.6	25.3 ± 2.6	0.83 ± 0.3	0

* Significantly different from control at $p < 0.05$.

Table 5. Malformation Index values of three phenol congeners in zebrafish embryos at 72-hpf.

		2 mg L^{-1}	**4 mg L^{-1}**	**8 mg L^{-1}**	**16 mg L^{-1}**	**32 mg L^{-1}**
	SP	1.8 ± 0.4	3.6 ± 0.5*	5.0 ± 1.0*	7.5 ± 1.0*	0
	2CP	2.0 ± 0.5	4.0 ± 0.6*	5.6 ± 0.6*	8.6 ± 1.6*	0
	2,4-DCP	2.5 ± 0.9	4.6 ± 0.5*	5.6 ± 1.0*	8.6 ± 1.0*	0
Control	1.7 ± 0.2					

* Significantly different from control at $p < 0.05$.

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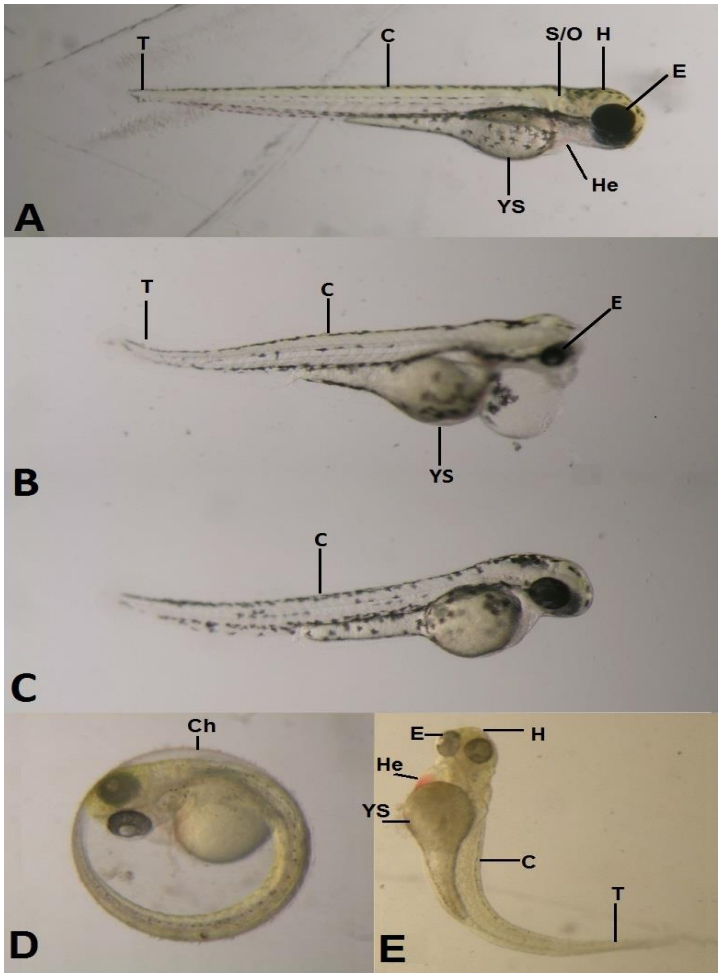

Fig 5. Photomicrograph of malformations in zebrafish larvae exposed to 16 mg L^{-1} SP, 8 mg L^{-1} 2CP and 4 mg L^{-1} 2,4-DCP. A) 72 hpf normal hatched larva with normal body structure, pigmentation and well-developed eyes. B) 72-hpf abnormal larva showing tail curvature, yolk sac edema and shrunken eyes. C) 72-hpf larva showed lordosis. D) Abnormal larva in chorion showed delayed hatching and weak pigmentation (72-hpf). E) Abnormal larva showing heart edema, tail curvature and non-pigmentation (72-hpf). C: chorda, Ch: chorion, E: eyes, H: head, He: heart, S/O: sacculus/otoliths, T: tail, YS: yolk sac.

DISCUSSION

Phenol and phenolic substances are considered as the main pollutant because of the toxic effects. The substances can also be accumulated in living organisms. Their contamination in aquatic system could pose potential threat to aquatic organisms. Therefore, the studies on the ecotoxicology of the chemicals are of important. In the current study, developmental toxicities of SP, 2-CP and 2,4-DCP were investigated by zebrafish bioassay. It was found that SP, 2-CP and 2,4-DCP had important effects on the early life stage of zebrafish embryos. One of the important effects was death. Other sub-lethal effects were incomplete eyes, head and tail, heart and chorda deformity, yolk sac edema, tail curvature, shrunken eyes, lordosis, delayed hatching, weak pigmentation, heart edema and depigmentation. The most toxic chemical to 48-hpf zebrafish embryo was 2,4-DCP, followed in decreasing order by 2CP and SP. Similar order of toxicity for the compounds on fish was shown in the literature. The toxic effect of SP can be understood by looking at its LC_{50} values which are less than others. For example, 96-h phenol LC_{50} value for 28-to 43-d-old *Oryzias latipes* was calculated as 38.3 mg L^{-1} (Holcombe *et al.* 1995). Also, the 48-h LC_{50} value of phenol for Japanese medeka (*Oryzias latipes*) was 24.1 mg L^{-1} (Rice *et al.* 1997). Fogels & Sprague (1977) reported that phenol (flow-through) 48-h LC_{50s} were 11.6 mg L^{-1} for rainbow trout, 30.9 mg L^{-1} for zebrafish and 36.3 mg L^{-1} for flagfish. Phenol (flow-through) 96-h LC_{50} value was 29 mg L^{-1} for 30-d-old fathead minnow (Phipps *et al.* 1981). In a static assay for 96-h, LC_{50} was determined as 40 mg L^{-1} for male *Poecilia reticulata* (Colgan *et al.* 1982). Moraes *et al.* (2015) found that the phenol LC_{50} values for 96-h were 15.08 and

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32.56 mg L^{-1} for *Ictalurus punctatus* (channel catfish) and *Piaractus mesopotamicus* (pacu) respectively. Our semi-static 48-h LC_{50} value of SP (substituted phenol) was 13.850 mg L^{-1} for 48-hpf zebrafish embryos and the value was consistent with the above literatures about fish.

In Figs. 2 and 3, the 48-h LC_{50} values obtained for 2CP and 2,4-DCP are lower than 10 mg L^{-1}. The results are consistent with the results of previous studies. In a previous study, it was shown that LC_{50} and EC_{50} values for 120-hpf zebrafish were 1.11, 2.45 mg L^{-1} and 0.74, 1.53 mg L^{-1} for 2,4,6-trichlorophenol and 2,4-DCP, respectively (Zhang *et al.* 2018). The 48-h LC_{50} values of 2CP and 2,4-DCP for fathead minnow (*Pimephales promelas*) were determined as 8.3 and 6.7 mg L^{-1} respectively (Blum & Speece 1991). The 24-h LC_{50} value of 2CP in goldfish was 16 mg L^{-1}, while 24-h LC_{50} value of 2,4-DCP was 7.8 mg L^{-1} in the same fish (Kobayashi *et al.* 1979). Small differences in LC_{50} values obtained from bioassays of acute toxicity tests may be due to various factors including fish life stage, fish body size, physicochemical properties of water, the absorption rate and detoxification mechanisms among species (Bucher & Hofer 1993; Rand *et al.* 1995; Saha *et al.* 1999). Fish are sensitive to various phenol concentrations ranging from 5.02 mg L^{-1} to 178 mg L^{-1}. The 96-hour LC_{50} value was 5.02 mg L^{-1} for rainbow trout and 2.5-h LC_{50} was 85 mg L^{-1} for goldfish (McLeay, 1976; Kishino & Kobayashi 1995). The 96-h LC_{50} values were determined in marine fish from 5.6 mg L^{-1} to 30.6 mg L^{-1} (Kondaiah & Murty 1994). The toxic effects of phenol on aquatic organisms were extensively studied. For example, 5- to 9-day LC_{50s} were 0.04 to 11.2 mg L^{-1} phenol for amphibians (Birge *et al.* 1980). Bernadini *et al.* (1996) reported that LC_{50} value was calculated as 178 mg L^{-1} phenol for *Xenopus* embryos.

Our results showed that the phenol compounds caused mortality and delayed hatching along with other malformations in zebrafish embryos. In a study related to zebrafish and chlorophenols, it was reported that the average number of spawned eggs of adult zebrafish exposed to 0.3 mg L^{-1} 2,4-DCP significantly decreased and it reduced hatching success of the fish eggs (Ma *et al.* 2012). Previously, Sawle *et al.* (2010) reported disruption of neurogenesis in zebrafish embryos with 2,4-DCP toxicity. In the same study, it was also reported that the 72-h LC_{50} and EC_{50} values of 2,4-DCP for zebrafish embryos were 38.9 and 10.8 µM, respectively. Phenols are also known to have lethal and teratogenic effects on other aquatic organism embryos. It was shown that phenols significantly reduced growth of larval fathead minnows at 0.25 mg L^{-1} and spawning at 0.62 mg L^{-1} concentrations (Dauble *et al.* 1983). In a previous study, it was observed that *Xenopus* embryos exposed to 5 mg L^{-1} of phenol grew more slowly and died in 3 weeks (Dumpert 1987). Paisio *et al.* (2009) showed that phenol produced teratogenic effects such as axial flexure, persistent yolk plug, irregular forms, acephalism, edema, axial shortening and different abnormalities in *Bufo arenarum* embryos (stage 25) at 150 mg L^{-1}, while lethal effects occurred at 183 mg L^{-1} after 96-h treatment. Bernadini *et al*. (1996) also reported that phenol caused serious malformations (generalized edema, intestinal and ocular malformations) in *Xenopus* embryos. These studies are in agreement with our teratogenic results.

Other toxicological effects of phenol compounds in several fish species were also reported. The toxic effects were haematological alterations in *Dicentrarchus labrax (*Roche & Boge 2000) and *Ictalurus punctatus* (Moraes *et al.* 2015), genotoxicity in *Scophthalmus maximus* (Bolognesi *et al.* 2006), carcinogenesis and mutagenesis in zebrafish (Yin *et al.* 2006), endocrine disruption in common carp (Kumar & Mukherjee 1988), and metabolism imbalance in *Brycon amazonicus* (Hori *et al.* 2006).

Phenols have adverse effects on aquatic life. Limited information is available regarding the mechanism of toxicity of phenols and their derivatives. It is known that phenols contribute to the loss of activity of some biochemical reactive enzymes. 2-CP induced reactive oxygen species (ROS) generation in fish (Luo *et al.* 2006). 2,4-DCP caused a range of oxidative damage both to proteins and lipids (Han *et al.* (1998). Also, phenol increases the formation of free radicals. The radicals reduce antioxidant capacity, leading to significant oxidative damage of important molecules such as DNA, protein and lipids (Murray *et al.* 2007). The pathway of phenol induced ROS generation was proposed as a mitochondrion NADH electron chain - dependent process and the negative pathway could lead to mitochondrion damage (Luo *et al.* 2008). The damage is most destructive to living organisms. It is considered that teratogenic fish embryos may be affected by phenols in the same way. However, other approaches are needed to explain developmental toxicity of phenols.

The pollution of phenol and their derivatives are common problems faced by worldwide population and ecosystems.

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Exceeding the standard levels in environment was not new issue for developed or developing countries (Gami *et al.* 2014). The studies of environmental levels of these and other phenols have been carried out for the last 30 years (Jin *et al.* 2012). The USEPA (1989) reported that the phenol level in environmental waters should not exceed 3.5 mg L^{-1} to protect human and animal health. At times phenols were determined at higher concentrations than these limits. For example, phenol concentrations ranging from 0.4 to 2.28 mg L^{-1} were found in some river waters (Paisio *et al.* 2009). This study showed both mortality and teratogenic effects at ecologically-relevant SP concentrations (8 and 16 mg L^{-1}). USEPA (1980b) reported chlorophenol concentrations ranging between 68 and 125 mg L^{-1} with the 2,4-DCP content ranging as high as 89% of the total in manufacturing effluent areas. Also, House *et al.* (1997) reported that concentrations ranging from <1 to 4.7 µg L^{-1} of 2,4-DCP in surface waters were determined in several countries. According to the measured concentrations of 2,4-DCP in the ecosystem and our selected concentrations of 2CP and 2,4-DCP, aquatic organisms should not be affected by the compounds now. The experimental concentrations of 2CP and 2,4-DCP used in the current study caused significant toxicity in zebrafish embryos after 48-h exposure. This study showed that phenols caused developmental toxicity in zebrafish, and the other toxic effects should be considered in future pollution research.

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چکیده

حضور مواد سمی در بوم سازگان، خصوصاً محیط‌های آبی به طور فزاینده‌ای در سال‌های اخیر افزایش یافته است. مشتقات فنل و فنل کلرینه جزء ترکیبات صنعتی سمی به حساب می‌آیند. فنل‌ها و مشتقات آن‌ها جزء آلاینده‌های محیطی محسوب می‌شوند. در این مطالعه، ۲و ۴ دی کلروفنل و ۲ کلروفنل و فنل جایگزین از نظر سمیت جنینی و تلفات در یک دوره چهار روزه با استفاده از جنین‌های زبرافیش، دانیو رریو آزمایش شدند. مشتقات فنلی مورد آزمایش سبب ناقص الخلقه شدن و تلفات جنینی شدند. غلظت کشنده میانی ۴۸ ساعته با جریان نیمه ساکن برای فنل جایگزین ۱۳/۸۵۰ میلی‌گرم در لیتر بود. همین غلظت برای ۲ کلروفنل و ۲و۴ دی کلروفنل به ترتیب ۸/۳۷۸ و ۶/۵۵۸ میلی‌گرم در لیتر بود. علایم نهایی شامل ناکامل شدن چشم‌ها، سر و دم، قلب و ناهنجاری طناب مازه (کوردا)، خیز در کیسه زرده، انحنای دم، چشمان فرورفته، انحراف عمودی ستون فقرات، تاخیر در تخم‌گشایی، کمرنگ شدن رنگدانه‌ها، خیز قلب و عدم شکل‌گیری رنگدانه‌ها پس از مواجهه با این ترکیبات بود. دیده شد که ۲و ۴ دی کلروفنل سمی‌تر از ترکیبات دیگر است. این مقاله اولین مورد از سمیت نسبی ترکیبات فنل در مراحل اولیه زندگی زبرافیش است.

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