Proc. Estonian Acad. Sci. Chem., 1997, **46**, 3, 130–139
https://doi.org/10.3176/chem.1997.3.06

A STUDY OF THE TOXICITY OF THE OZONATION PRODUCTS OF PHENOLS AND CHLOROPHENOLS BY *Daphnia magna* TEST

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Received 22 April 1997, revised 4 June 1997, accepted 9 June 1997

Abstract. Ozonation was found to be quite effective for the destruction of substituted phenols, chlorophenols, and resorcinols in water, and also phenols and chlorophenols containing waste water. The toxicity of ozonation products was assayed by *Daphnia magna* 24 h toxicity test. The toxicity of ozonation products of eight individual chlorophenols and their mixture was ascertained to be significantly lower than the toxicity of unozonated solutions of chlorophenols. However, for some other phenols under study the toxicity increased during the ozonation and the formation of very toxic products was observed. Therefore, the toxicity of the ozonated samples was significantly higher than for unozonated ones.

Key words: phenols, chlorophenols, ozonation, *Daphnia magna*, toxicity.

INTRODUCTION

Phenols are a widespread class of water pollutants. A high level of phenols in a waste water may be produced by a number of fuel conversion processes, such as oil refining, coke manufacture, coal conversion, shale oil recovery, etc. Chlorophenols are widely used in the manufacture of pesticides, insecticides, herbicides, fungicides, intermediates of dyes, and in various organic syntheses. Chlorination of phenols during the disinfection of raw water, waste water, and bleaching in the paper industry also produces chlorophenols.

Phenols and chlorophenols can be degraded by bacteria. However, as phenols and chlorophenols are highly toxic, the conditions for biological degradation are often unfavourable. Chemical oxidation can be an alternative method for the treatment of water containing phenols.

Ozone is highly reactive towards phenol, some substituted phenols, resorcinol [1–3], and chlorophenols [4–6]. It has been ascertained that the process of the ozonation of phenols is dependent upon the acidity of water. Evidently, under suitable conditions phenols may be degraded by ozonation quite successfully.

However, the target of water and waste water treatment is not only to destroy the pollutants, but mainly to reduce the toxicity or increase the biodegradability of water, i.e. to make water less toxic and, thus, more friendly to the environment. Therefore, the toxicity of the ozonation by-products is extremely important. The aim of the present study was to estimate the toxicity of the by-products of ozonation performed in aqueous media for different individual phenols and chlorophenols and their mixtures.

MATERIALS AND METHODS

The experiments on the ozonation of phenols were performed in a semibatch bubble column into which ozone gas was supplied continuously. A mixture of ozone gas and air was generated by compressing air through the laboratory ozone generator to a bubble column, 264 ml in volume (21 cm in height and 4.35 cm in diameter; inside the bubble column there was a quartz tube, 17 cm in height and 1.9 cm in diameter). The gas flow rate was kept 1 l/min. The ozone concentration in the feed-gas and in the off-gas was measured spectrophotometrically ($\lambda = 258$ nm) using a Specord UV/VIS (Carl Zeiss, Jena, GDR) spectrophotometer. The ozone concentration in the feed-gas was kept at 0.50 ± 0.02 mg/l. The concentration of ozone dissolved in water was measured by the indigo method [7]. All chemicals were analytical grade. The samples were prepared by dissolving the phenols in distilled water. A standard procedure was ozonation of 100 ml of fresh phenol solution using different treatment times. After the selected reaction time, the ozonated sample was taken out of the reactor for further analyses. The ozonation times were selected so that during the longest ozonation at least about 90% reduction of the initial phenol was achieved.

The initial concentrations of phenols in the experiments with individual phenols were 0.4 mM and in the mixtures 1 mM (0.2 mM of each for the mixture of chlorophenols and 0.1 mM of each for the mixture of substituted phenols). The solutions were ozonated both in the acidic and basic media. The pH in the acidic medium was 2.5 and in the basic medium 9.5. For the adjustment of pH 0.1 and 1.0 N H_2SO_4, or 0.2 N and 0.1 N NaOH were used.

The concentrations of phenols during the experiments were measured with a high performance liquid chromatograph (Millichrom, Nauchpribor, USSR) equipped with a UV spectrophotometer (190–360 nm). A reverse phase column, packed with Separon C18 (Chemapol, Czechoslovakia), 64 mm in length and 2 mm in diameter, was applied. The isocratic method with a solvent mixture of

50% acetonitrile and 0.1% acetic acid in water was applied (the flow rate was 100 µl/min). The wavelength used in the UV-detector was 220 or 230 nm.

In the acute toxicity tests less than 24 h old specimens of *Daphnia magna*, obtained from a single culture vessel, were used. Three replicates and a control provided data on 20 animals. These tests exposed the organisms to ten (dilution series from 5 to 90% effluent) effluent concentrations and a control. The median effective concentration (EC_{50}) values were determined according to the Finnish Standard SFS 5062 [8], using a special computer program PROBIT accepted for this standard. The calculated EC_{50} is based on the data on 165 animals (including control) for each experimental point. The acute toxicity of ozonation products of phenols was compared with the toxicity of pure phenols. The toxicity of pure phenols was evaluated calculating EC_{50} values and 95% confidence limits from the results obtained from these 24 h toxicity tests. All toxicity tests were duplicated. The duration of ozonation was selected so as to achieve at least 90% conversion of the initial phenol into products. The initial concentration used in the experiments was 0.4 mM. The *Daphnia* clone used was of Finnish origin (*Daphnia magna* EF) from the North Savo Regional Environmental Centre in Kuopio. The living conditions were kept optimal according to [8]. In all experiments the mortality in control tubes was 0%.

RESULTS AND DISCUSSION

Data on the ozonation of phenols are presented in Table 1. Our results prove that ozonation is a powerful method for the destruction of various phenols. The analysis of the experimental data showed that the decrease in the concentration of all phenols during ozonation followed the pseudo-first order kinetics (the squared correlation coefficients R^2 were 0.94–0.99) in both media. The half-lives of chlorophenols, phenol, xylenols, cresols, and resorcinols were found to be a few minutes or even less when ozonation was conducted in the basic medium. When ozonation was conducted in the acidic medium the ozonation rate was slower but still quite significant (see Table 1). In general, the efficiency of ozonation in the acidic medium (pH 2.5) followed the order 4CP < 6CCR < P < 2CP = RES < 4CRES = 2,4DCP < 2,3DMP = 2,4,6TCP ≤ 2CR < 2,6DMP = 2,5DMP ≤ 5MRES < 3,4DMP ≤ 2,3,4,6TeCP ≤ 4CR < 2,4DMP. The corresponding 90% conversion times fell from 63.3 min for 4-chlorophenol to 8.2 min for 2,4-dimethylphenol. In the basic medium the 90% conversion times were quite close for different phenols and did not exceed 5 min of the ozonation.

The main target of the investigation was to determine whether ozonation of phenols can decrease the toxicity of the solutions. The corresponding data are presented in Figs. 1–3. The data indicate that quite significant changes take place in the toxicity of the solutions during ozonation.

Table 1

Efficiency of the ozonation of phenols

Phenol	Initial pH	90% destruction time, min	Ozone dose, mg/l	
			applied	consumed
Phenol (P)	2.5	50.6	248.0	48.0
	9.5	4.0	21.0	16.5
o-Cresol (2CR)	2.5	23.5	118.0	26.3
	9.5	3.4	17.2	14.4
p-Cresol (4CR)	2.5	12.4	62.0	17.9
	9.5	2.7	13.5	9.6
2,3-Xylenol (2,3DMP)	2.5	24.8	119.0	34.7
	9.5	2.0	9.6	9.6
2,4-Xylenol (2,4DMP)	2.5	8.2	40.9	15.8
	9.5	2.95	14.8	14.5
2,5-Xylenol (2,5DMP)	2.5	19.0	91.2	23.5
	9.5	2.15	10.8	10.8
2,6-Xylenol (2,6DMP)	2.5	19.4	94.0	23.0
	9.5	2.0	10.0	10.0
3,4-Xylenol (3,4DMP)	2.5	13.7	68.5	19.6
	9.5	2.4	12.0	11.8
2-Chlorophenol (2CP)	2.5	48.2	231.0	25.3
	9.5	4.1	20.5	19.2
4-Chlorophenol (4CP)	2.5	63.3	328.0	43.9
	9.5	4.0	20.8	19.8
2,4-Dichlorophenol (2,4DCP)	2.5	39.3	206.0	37.0
	9.5	3.4	17.0	16.3
2,4,6-Trichlorophenol (2,4,6TCP)	2.5	24.9	120.0	23.9
	9.5	4.1	20.9	17.0
2,3,4,6-Tetrachlorophenol (2,3,4,6TeCP)	2.5	13.0	66.3	18.4
	9.5	4.5	21.6	17.3
Pentachlorophenol (PCP)	9.5	3.4	16.7	15.6
4-Chlororesorcinol (4CRES)	2.5	40.8	208.0	30.0
	9.5	5.0	25.0	24.0
6-Chloro-*m*-cresol (6CCR)	2.5	53.5	268.0	45.5
	9.5	4.4	22.0	18.3
Resorcinol (RES)	2.5	47.8	248.0	49.0
	9.5	4.7	23.4	17.5
5-Methylresorcinol (5MRES)	2.5	18.7	93.4	21.6
	9.5	3.1	15.6	15.4

The toxicity of the ozonation products of eight chlorophenols was significantly lower than the toxicity of unozonated solutions of chlorophenols. The ozonation of chlorophenols led to continuous decrease in the toxicity of the phenolic solutions independent of pH of the initial solution. The samples became less toxic when the chlorophenols were destroyed. The ultimate detoxication of the solution was achieved during a period that did not exceed the 90–95% conversion time of the initial chlorophenol (see Table 2). A similar behaviour was observed when ozonation was applied to a mixture of chlorophenols. A continuous detoxication followed the decrease in the concentrations of chlorophenols during ozonation (see Fig. 1). Therefore, ozonation may be recommended as an effective method for the destruction and detoxication of chlorophenols.

Table 2

Toxicity of untreated and ozonated chlorophenols

Phenol	EC_{50} and 95% confidence limits, mg/l, untreated phenols	EC_{50} for ozonated to 90–99% conversion of initial phenol, % of sample
2-Chlorophenol	21.5 (20.0–23.0)	n.t.[1] a[2] n.t. b[3]
4-Chlorophenol	12.8 (10.9–14.6)	n.t. a n.t. b
2,4-Dichlorophenol	6.7 (6.0–7.0)	n.t. a n.t. b
2,4,6-Trichlorophenol	13.0 (11.7–13.8)	n.t. a n.t. b
2,3,4,6-Tetrachlorophenol	8.8 (8.0–9.8)	n.t. a n.t. b
Pentachlorophenol	3.6 (2.9–5.1)	n.t. a n.t. b
4-Chlororesorcinol	5.35 (4.65–6.0)	n.t. a n.t. b
6-Chloro-*m*-cresol	5.0 (3.8–5.9)	n.t. a n.t. b

[1] n.t., not toxic according to the *Daphnia magna* test; [2] a, ozonation in the acidic medium; [3] b, ozonation in the basic medium.

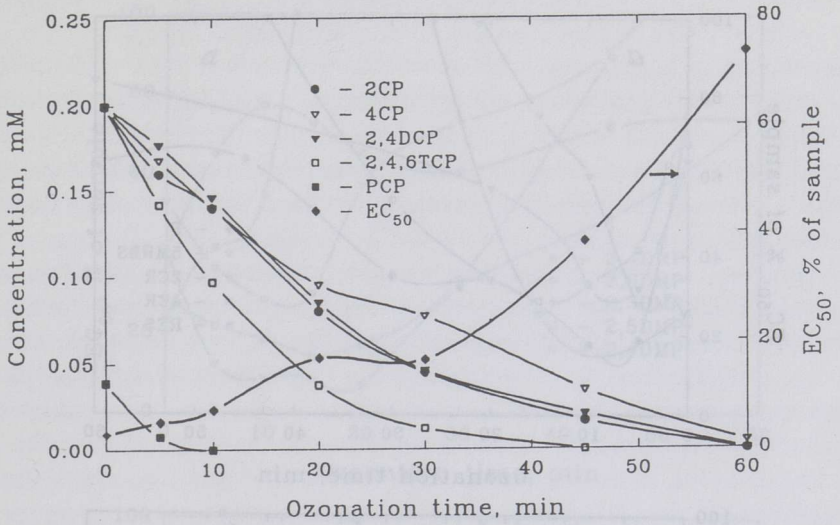


Fig. 1. Changes in the concentrations of chlorophenols and the EC_{50} value during the ozonation of the mixture of chlorophenols in the acidic medium (pH 2.5). The mortality in the control tubes was 0% in all experiments.

No formation of stable toxic products was observed during the ozonation of resorcinols (see the corresponding curve in Fig. 2). In both media ozonation led to the detoxication of the solution and the samples became nontoxic in quite reasonable time simultaneously with the destruction of the initial resorcinol and 5-methylresorcinol.

The situation was different when phenol, cresols, and dimethylphenols were ozonated. The decrease in the concentration of phenol and cresols did not lead to the corresponding decrease in the toxicity of the ozonated solution and the ozonation by-products turned out to be significantly more toxic than the initial phenol (see Fig. 3). Moreover, they proved to be quite steady towards further ozonation and detoxication could be achieved only when quite high ozone doses (and, correspondingly, long ozonation time) were applied. When ozonation was conducted in the basic medium these doses exceeded 2–5 times the doses required for the total destruction of the initial phenols.

The ozonation of some dimethylphenols also led to the formation of ozone-resistant by-products that were in some cases significantly more toxic than the initial phenols. When the EC_{50} values of the ozonated dimethylphenols were plotted versus the ozonation time for two different ozonation procedures (i.e. in the acidic and basic media) the curves were definitely different for the two procedures for all dimethylphenols except 2,4-dimethylphenol (cf. Fig. 3). For

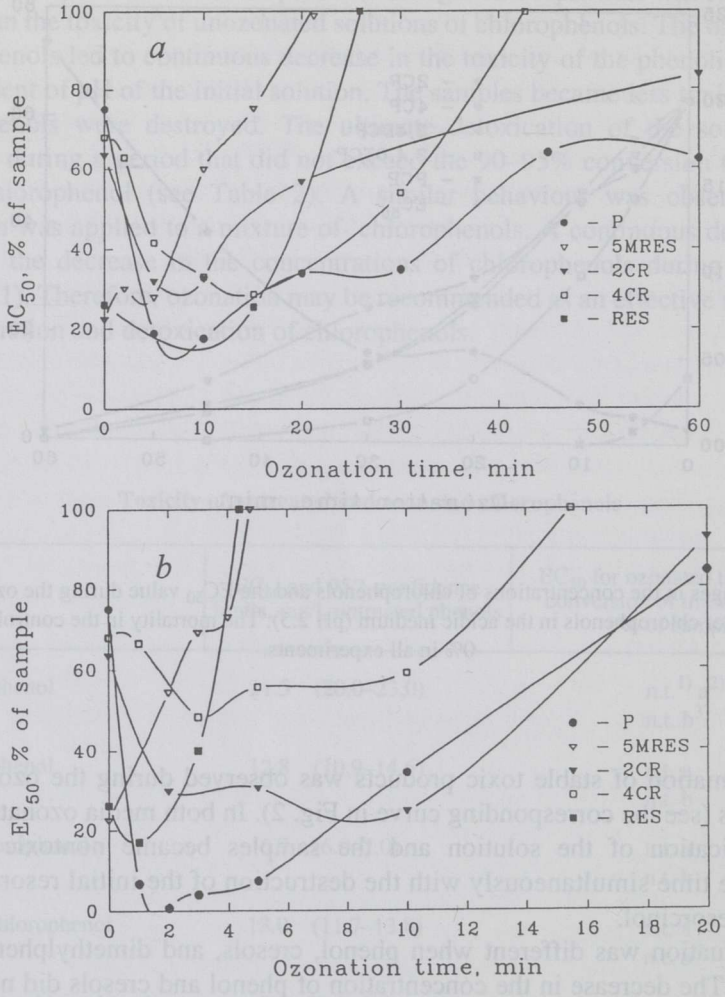
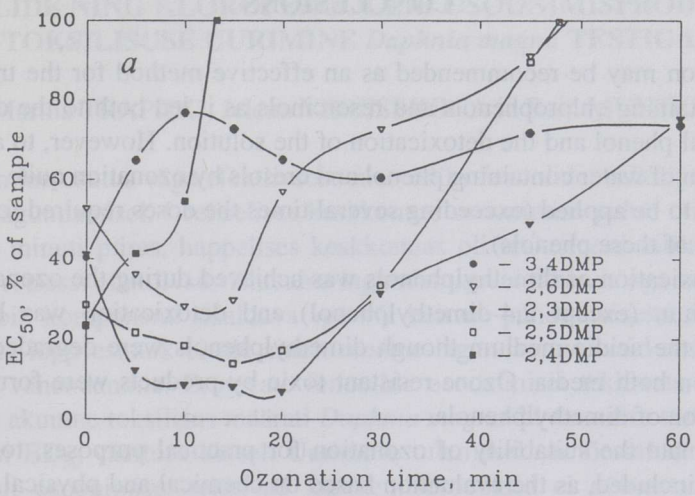


Fig. 2. Changes in the EC_{50} value during the ozonation of phenols. *a*, acidic medium (pH 2.5); *b*, basic medium (pH 9.5).

2,4-dimethylphenol detoxication was achieved during ozonation along with its degradation. For 2,3-; 2,5-; and 2,6-dimethylphenols in the basic medium no detoxication was achieved even when long ozonation times (and correspondingly high ozone doses) were used and the initial phenol was destroyed completely. Moreover, the toxicity increased during the ozonation, and was significantly higher for the ozonated samples (the ozone dose applied exceeded three times or more the dose required for the total destruction of the corresponding dimethyl-



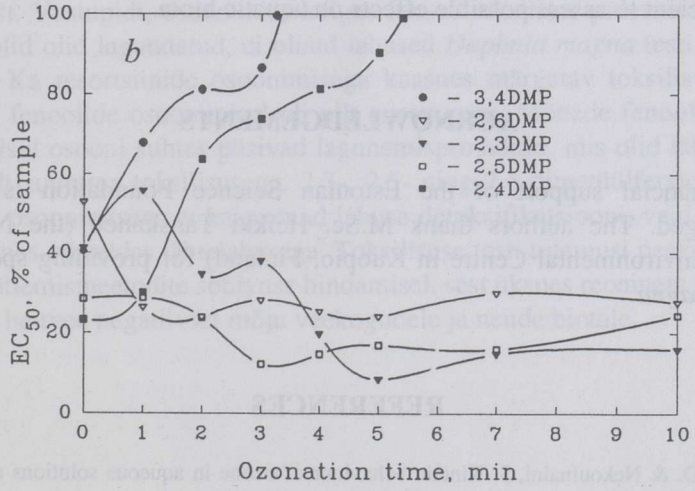


Fig. 3. Changes in the EC_{50} value during the ozonation of dimethylphenols. *a*, acidic medium (pH 2.5); *b*, basic medium (pH 9.5).

phenol) than for the unozonated ones. In the acidic medium a reduction of the toxicity occurred during further ozonation of these dimethylphenols. In general, the detoxication was quite slow and required high ozone doses to be applied.

Although dimethylphenols were degraded quite easily by the ozonation in both media, ozonation cannot be regarded as an effective method of the treatment of dimethylphenols (except 2,4-dimethylphenol) as detoxication – the main goal of the treatment – was not achieved.

CONCLUSIONS

Ozonation may be recommended as an effective method for the treatment of wastes containing chlorophenols and resorcinols as it led both to the degradation of the initial phenol and the detoxication of the solution. However, to achieve the detoxication of water containing phenol and cresols by ozonation quite high ozone doses have to be applied (exceeding several times the doses required for complete destruction of these phenols).

No detoxication of dimethylphenols was achieved during the ozonation in the basic medium (except 2,4-dimethylphenol) and detoxication was hard to be attained in the acidic medium though dimethylphenols were degraded easily by ozonation in both media. Ozone-resistant toxic by-products were formed during the ozonation of dimethylphenols.

To estimate the suitability of ozonation for practical purposes, toxicity tests have to be included, as the evaluation based on chemical and physical tests alone is not sufficient to assess possible effects on aquatic biota.

ACKNOWLEDGEMENTS

The financial support of the Estonian Science Foundation is gratefully acknowledged. The authors thank M.Sc. Heikki Tanskanen (the North Savo Regional Environmental Centre in Kuopio, Finland) for providing specimens of *Daphnia magna*.

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FENOOLIDE NING KLOROFENOOLIDE OSOONIMISPRODUKTIDE TOKSILISUSE UURIMINE *Daphnia magna* TESTIGA

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Osoonimine osutus väga efektiivseks meetodiks fenoolide, eelkõige asendatud fenoolide lagundamisel. Leeliselises keskkonnas osoonimise puhul olid poolitusajad mõne minuti piires, happelises keskkonnas oli reaktsiooni kiirus tunduvalt väiksem. Protsessi efektiivsus lähteaine lagundamisel ei tähenda aga veel, et sama meetod sobib komponenti sisaldava vee või heitvee puhastamiseks, sest heitvee puhastamise lõppeesmärk ei ole ainult reoaine lagundamine, vaid eeskätt lahuse toksilisuse vähendamine. Erinevate fenoolide osoonimisel tekkivate lagunemisproduktide akuutne toksilisus määrati *Daphnia magna* 24-tunnilise testi abil efektiivse doosi ED_{50} väärtuse alusel. Uurimuse tulemusel on tõestatud, et kaheksa klorofenooli osoonimisel tekkivad lagunemisproduktid ei põhjusta toksilisuse suurenemist. Vastupidi, osoonimise käigus lahuse toksilisus pidevalt langes. Kui klorofenoolid olid lagundatud, ei olnud lahused *Daphnia magna* testi järgi enam toksilised. Ka resortsiinide osoonimisega kaasnes märgatav toksilisuse langus. Asendatud fenoolide osoonimisel oli pilt teistsugune. Mõnede fenoolide osoonimisel tekkisid osooni suhtes püsivad lagunemisproduktid, mis olid lähtefenoolist märgatavalt suurema toksilisusega. 2,3-; 2,5- ning 2,6-dimetüülfenoolide küllalt pikaajalise osoonimisega ei kaasnenud lahuse detoksifikatsiooni, vaid selle toksilisus suurenes võrreldes lähtelahusega. Toksilisuse testi tulemusi peaks arvestama heitvee töötlemismeetodite sobivuse hindamisel, sest üksnes reoainete lagunemine ei hoia ära heitvee negatiivset mõju veekogudele ja nende biotale.