Decomposition of Tetrachloro-1,4-benzoquinone (p-Chloranil) in Aqueous Solution

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Introduction

p-Chloranil (Figure 1) has been observed as a significant product from the oxidation of pentachlorophenol (PCP) when mediated by phenol-oxidizing enzymes in vitro (1–3), by fungal metabolism (4, 5) and by TiO₂-assisted photolysis (6). In the past, p-chloranil was used as a fungicide and algicide under the trade name Spargan (7). It is known to form protein adducts both in vitro and in vivo and has been implicated in the genotoxicity associated with PCP (8). While there is much interest in developing technologies to remediate PCP-contaminated soils and groundwater (9, 10), the formation of toxic byproducts such as chloranil must be considered and, if possible, overcome.

Work reported in the 1960s (11, 12) indicated that chloranil is hydrolyzed in strong alkaline solution to yield chloranilic acid (2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone; Figure 1) through an intermediate designated trichlorohydroxyquinone (TrCHQ; Figure 1). Under milder conditions in aqueous solution, the colorless chloranil can be observed to develop a purple color (2), suggesting that decomposition reactions may occur in systems of environmental interest. The purposes of the current study were to identify TrCHQ and chloranilic acid as products of chloranil hydrolysis at near-neutral to slightly acidic pH, to quantify the kinetics of chloranil and TrCHQ decomposition, and to evaluate the potential acute aquatic toxicity of chloranil and its decomposition products using the Microtox screening assay. Because chloranil is formed from PCP in peroxidase-catalyzed oxidation reactions (2, 3), we also examined the influence of hydrogen peroxide on chloranil decomposition.

Materials and Methods

Chemicals. p-Chloranil (99%) and chloranilic acid (99%) were purchased from Sigma Chemical Co. (St. Louis, MO). Stock solutions of each compound were prepared gravimetrically in methanol and stored at 4 °C. Hydrogen peroxide stock was prepared by dilution of 30% reagent-grade hydrogen peroxide and was calibrated by titration against potassium permanganate standard in 1 N H₂SO₄. Buffers (sodium tartrate for pH 3–5, sodium or potassium phosphate for pH 6–9, and ammonium hydroxide for pH 10) were prepared from reagent-grade acids and salts. Ethyl acetate was reagent-grade, and all other solvents were high-pressure liquid chromatography (HPLC) or spectrophoto metric grade. ¹⁸O-Labeled water (95 atom % ¹⁸O) was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Determination of Molar Extinction Coefficients. The molar extinction coefficients at 530 nm for both chloranilic acid and TrCHQ in water were determined by measuring absorbance as a function of concentration using a Hitachi U2000 double-beam spectrophotometer. TrCHQ was prepared by mixing known concentrations of reagent chloranil in pH 8.5 buffer and waiting until a stable value of A₅₃₀ was reached. Complete loss of chloranil under these conditions was confirmed by HPLC, and stoichiometric

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conversion to TrCHQ was assumed based on the appearance of a single peak in HPLC analysis, the area of which correlated linearly with initial chloranil concentration \((n^2 = 0.99, n = 4)\). The estimated \(k_{1,obs}\) of TrCHQ was \(1.41 \times 10^3\) L mol\(^{-1}\) cm\(^{-1}\) \((n = 0.98; n = 5)\), which agrees well with the reported value of \(1.45 \times 10^3\) at 535 nm \((11)\), and was unaffected by pH over the range 6.9–8.5 (the range used in kinetic studies). The measured \(k_{2,obs}\) for chloranilic acid was \(176\) L mol\(^{-1}\) cm\(^{-1}\) at pH 7.0, increasing slightly as pH decreased and reaching a value of \(227\) L mol\(^{-1}\) cm\(^{-1}\) at pH 4.1.

**Analysis of Decomposition Products.** Chloranil solutions (100 mL at 30 \(\mu\)M) were prepared in buffers ranging from pH 7 to pH 10 and allowed to decompose for 24 h at room temperature (approximately 23 °C). Each solution was acidified with HCl to pH 1 and then extracted with two successive 10 mL volumes of ethyl acetate. The ethyl acetate was evaporated under a stream of nitrogen gas, and the extracted material was redissolved in 90% methanol/10% water (v:v) to a final volume of 1 mL immediately prior to electrospray mass spectrometry (ES/MS) analysis. A chloranilic acid standard (3 mM) was prepared in 90% acetone/10% water and in 90% acetone/10% water (v:v). ES/MS was performed by flow injection of each solution at a rate of 3 \(\mu\)L/min. Mass spectra were obtained in the negative ion mode on a Finnigan 4000 quadrupole mass analyzer retro-fitted with an Analytica of Branford, Inc. electrospray source. Nitrogen was used as the drying gas and was heated to 200 °C, and SF6 was used as the sheath gas. For each solution, the inlet capillary was held at ground, and the cylindrical, backing, and capillary electrodes were held at 2500, 2800, and 3000 V, respectively. For the chloranil standards, the inlet capillary was held at −2500 to −3500 V, and the cylindrical and backing electrodes were held at ground. The mass analyzer was scanned from 10 to 500 amu, and the data were collected on a Technivent Vector 2 data system. Chloranil was also analyzed by full-scan (50–650 amu) electron-impact ionization analysis on a VG 70 SEQ hybrid mass spectrometer, after introducing chloranil into the mass spectrometer using a direct-insertion probe.

HPLC analysis of chloranil and chloranilic acid was performed with a Waters 600E system and Millennium data collection and operating software. Chloranil was analyzed under isocratic conditions using a C18 column with a mobile phase (1 mL/min) consisting of 60:40 methanol/water (v:v) plus 0.04% trifluoroacetic acid and UV detection at 254 nm. Chloranilic acid was analyzed by C18 isocratic HPLC with detection at 320 nm, using a mobile phase of 30:70 methanol-buffered water (12.5 mM potassium phosphate, pH 6.8) containing 5 mM of the ion pairing reagent tetrabutylammonium hydrogen sulfate (TBAS; Aldrich). Both chloranil and chloranilic acid were quantified by external standardization against the reagent-grade chemicals.

Qualitative HPLC analysis to verify the existence of decomposition products generated in the presence of methanol (in pH 8.5 buffer) was conducted under isocratic conditions using a mobile phase of 50:50 methanol-buffered water containing TBAHS as described above and visible detection at 530 nm. Under these conditions, chloranilic acid eluted as an unretained peak, but two overlapping peaks were separated from chloranilic acid and from TrCHQ. The absence of these peaks in mixtures from which methanol was excluded was determined by adding solid chloranil directly to the pH 8.5 buffer and analyzing the aqueous phase over time by the same HPLC method.

The yield of chloranilic acid from chloranil was determined by incubating triplicate samples of 30 \(\mu\)M chloranil in pH 8.5 buffer. After 5 d of incubation, chloranilic acid was quantified by HPLC, and a yield of 75% was calculated. The final absorbance at 530 nm of the 30 \(\mu\)M chloranil solution was 0.003 unit higher than if a 100% yield of chloranilic acid were achieved (the expected \(A_{340}\) of 30 \(\mu\)M chloranilic acid = 0.005), indicating that other products also contributed to absorbance at 530 nm.

**Aqueous Decomposition Kinetics.** Chloranil solutions (30 \(\mu\)M) were prepared in buffers at various pH, and absorbance at 530 nm was followed with time at room temperature. Chloranil was assumed to be converted to TrCHQ by hydrolysis as follows:

\[
C + OH^- \rightarrow k_{1,obs} TrCHQ + Cl^- \tag{1}
\]

where C is chloranil and \(k_{1,obs}\) is a pseudo-first-order rate coefficient at constant pH. The decomposition of TrCHQ in the presence of methanol was assumed to proceed as two parallel reactions:

\[
TrCHQ + OH^- \rightarrow k_{2,obs} CA + Cl^- \tag{2}
\]

\[
TrCHQ + MeOH \rightarrow k_{3,obs} other\ products \tag{3}
\]

where CA is chloranilic acid, and \(k_{2,obs}\) and \(k_{3,obs}\) are pseudo-first-order rate coefficients at a given pH and fixed methanol concentration for reactions 2 and 3, respectively. The other products are those observed by HPLC in reactions containing methanol introduced with the chloranil stock solution; the methanol concentrations in these reactions were far higher than the initial chloranil concentration and, therefore, could be treated as constant. The two pseudo-first-order reactions shown as eqs 2 and 3 were combined and expressed as an effective first-order reaction at a fixed pH:

\[
TrCHQ \rightarrow k_{2,eff} CA + \text{other products} \tag{4}
\]

where \(k_{2,eff}\) is the effective first-order rate coefficient \((=k_{2,obs} + k_{3,obs})\) for the decomposition of TrCHQ at constant pH in the presence of excess methanol.

For two first-order reactions in series (reactions 1 and 4), the concentration of the intermediate (TrCHQ) can be expressed as a function of time in a batch system (16):

\[
[TrCHQ] = \left(\frac{k_{1,obs}}{k_{2,eff} - k_{1,obs}}\right)[C]_0(e^{-k_{1,obs}t} - e^{-k_{2,eff}t}) \tag{5}
\]

The total concentration of products is given by (16):

\[
[CA + \text{other products}] = [C]_0 \left\{1 - \frac{k_{2,eff}e^{-k_{1,obs}t} - k_{1,obs}e^{-k_{2,eff}t}}{k_{2,eff} - k_{1,obs}}\right\} \tag{6}
\]

At any time, the absorbance at 530 nm represented the sum of the contributions of TrCHQ, chloranilic acid, and other products, or assuming equivalent minor contributions from chloranilic acid and other products (see above):
\[ A_{330} = \epsilon_{530,TrCHQ}[TrCHQ] + \epsilon_{530,CA}[CA + \text{other products}] \]

By substituting eq 5 for [TrCHQ], eq 6 for the total concentration of products, and the measured extinction coefficients for TrCHQ and chloranilic acid, an equation relating \( A_{330} \) vs time was obtained. This equation was fit to the data using non-linear regression to obtain best estimates for both \( k_{1,\text{obs}} \) and \( k_{2,\text{eff}} \) at each pH value tested. Nonlinear regression was conducted with SYSTAT (SYSTAT, Inc., Evanston, IL).

To account for possible influences of the buffer on the observed changes in absorbance, chloranil was incubated at pH 7.6 in the presence of phosphate buffer at concentrations of 1, 10, and 100 mM (triplicate reactions at each concentration), and the absorbance at 530 nm was measured after 65 min. Another set of reactions was run at pH 8.8 in the presence of carbonate buffer or phosphate buffer (triplicate reactions for each buffer), and absorbance was measured after 30 min. The effect of oxygen on the rate of chloranil decomposition was evaluated in side-by-side reactions containing 30 µM chloranil in pH 7 buffer. Triplicate reactions were run in air-saturated buffer, and triplicate reactions were run headspace-free in buffer that had been purged with helium. Optical absorbance at 530 nm was then followed at selected intervals over a 3-h reaction period.

Stoichiometry of the Reaction with Hydrogen Peroxide.
The stoichiometry of hydrogen peroxide consumption from its reaction with chloranil was determined by adding peroxide (100 µM) to solutions of chloranil at pH 7. Residual peroxide was measured after 10 min or 10 h using an enzymatic assay. The enzymatic assay involved adding a small aliquot of sample to a mixture containing Coprinus macrorhizus peroxidase (CMP; a research gift from Novo Nordisk A/S, Bagsvaerd, Denmark) at 1 µg/mL and 1.7 mM peroxidase substrate ABTS (2,2'-azinobis[3-ethylbenzothiazoline-6-sulfonic acid]), diaminomutual salt: Sigma) in a pH 6 phosphate buffer. ABTS is converted in this assay to the chromophore ABTS+ (I

The combined yield of TrCHQ and chloranilic acid was estimated in triplicate samples in which peroxide (100 µM) and chloranil (50 µM) were mixed at pH 7 for 4 h. The pH of the samples then was raised to 10 to hydrolyze any TrCHQ that might have formed. Total chloranilic acid concentration was then determined by HPLC.

Kinetics of Reaction with Hydrogen Peroxide.
Chloranil solutions (30 µM) were prepared in buffers at different pH and mixed with hydrogen peroxide (120 µM). Aliquots of each mixture were treated with catalase to terminate the reaction at specific time intervals and then analyzed by HPLC for residual chloranil concentration. Concentration vs time data were used to estimate rates of chloranil consumption at each pH by nonlinear regression using a first-order decay model.

The effect of peroxide concentration on reaction rate was determined at pH 4.1 and a nominal initial chloranil concentration of 50 µM. Reaction mixtures were prepared directly in HPLC autosampler vials and injected at intervals programmed into the HPLC controller. Actual initial concentrations of chloranil used for kinetic analysis were those measured in the first injection, which were between 43 and 46 µM in all cases. Data from each set of injections corresponding to a given hydrogen peroxide concentration were analyzed according to a first-order decay model.

Toxicity Analysis.
The Microtox acute toxicity screening assay was conducted using procedures described in detail elsewhere (18). Chloranil samples were prepared in pH 7 buffer and analyzed immediately or were allowed to decompose for 3.5 h before beginning the Microtox analysis. Solutions containing chloranil plus an equimolar amount of hydrogen peroxide in pH 7 buffer were mixed for 80 min before analysis. Chloranilic acid and PCP samples were prepared in pH 7 buffer from standard solutions. EC50 values (concentrations corresponding to 50% attenuation in light output by the luminescent test organism) were determined by instrument software (Microbics Corp., Carlsbad, CA).

Results and Discussion
Aqueous solutions of p-chloranil will develop a faint purple color when left standing at near-neutral pH and at room temperature (2), indicating that hydrolysis reactions previously reported to occur under strongly alkaline conditions (11) might also occur, albeit more slowly, under environmentally relevant conditions. Preliminary efforts to analyze for the presence of TrCHQ and chloranilic acid by gas chromatography/mass spectrometry (GC/MS) were unsuccessful because of the high polarity of these compounds. Accordingly, we analyzed decomposed chloranil samples by flow injection ES/MS. The mass spectrum of a sample at pH 8 for 24 h is shown in Figure 2, along with mass spectra of standards of chloranil and chloranilic acid.

The mass spectrum of the chloranil standard in acetonitrile (Figure 2a) is characterized by a four-chlorine isotope cluster at m/z 244 and a three-chlorine isotope cluster at m/z 225. The three-chlorine cluster evident at m/z 225 presumably is the M-H via TCHQ, since we cannot postulate a fragmentation mechanism for chloranil from which such a cluster could be observed. The appearance of TrCHQ in the chloranil standard was inferred to result from reactions occurring during analysis and not due to contamination of the standard, based on several observations. We did not observe the presence of TrCHQ in the electron-impact mass spectrum of chloranil, and complex patterns of three-chlorine and four-chlorine isotope clusters beginning at m/z 225 and m/z 244, respectively, were observed in the ES mass spectra obtained from chloranil dissolved in 90% acetonitrile/10% H2O (not shown). These patterns were indicative of mixed 18O and 18O isotopes of the hydroxyl group in TrCHQ and also the carbonyl oxygen.
FIGURE 2. ES mass spectra of (a) chloranil; (b) chloranilic acid; (c) extract from chloranil sample allowed to decompose for 24 h at pH 8.

in both TrCHQ and chloranil (such exchange of an aromatic carbonyl oxygen with that in water has been reported to occur for 2,6-dichloro-1,4-benzoquinone [2]). The presence of a hydroxyl oxygen derived from water indicates that chloranil was hydrolyzed partially during analysis. It is possible that, during electrospray, the enrichment of OH− ions during droplet disintegration and evaporation of the solvent create a basic environment that is sufficient to hydrolyze chloranil to TrCHQ.

Because little fragmentation occurs during ES/MS, spectra corresponding to the deprotonated chloranilic acid molecular ion (m/z 207) and to the deprotonated TrCHQ molecular ion (m/z 225) are distinguishable in the decomposed chloranil sample at pH 8 (Figure 2c). The intensity of the TrCHQ (M − H)− ion decreased with increasing pH and corresponded to an increasing intensity of the chloranilic acid (M − H)− ion (not shown), illustrating that the rate of chloranilic acid formation from chloranil increased with increasing pH (all samples were incubated for the same period of time).

The spectra shown in Figure 2c also include a smaller two-chlorine isotope cluster at m/z 221, which would be consistent with a methyl ether of chloranilic acid or an equivalent compound. The existence of such a product derived from TrCHQ is consistent with HPLC analysis, in which two overlapping peaks observed in reaction mixtures containing methanol were not observed when solid chloranil was used instead of the methanol stock; these peaks might represent isomers resulting from the displacement of chloride by the methoxy group at different positions in TrCHQ. Based on the measured yield of chloranilic acid from the overall hydrolytic decomposition of chloranil, the methoxy-substituted product(s) account for 25% or less of the original chloranil on a molar basis.

Kinetics of Hydrolysis Reactions. Solutions of chloranil were prepared in buffers over a pH range from 4 to 8.1, and absorbance at 530 nm was followed over time. At pH 7 and above, a rapid increase in A530 was observed within 2 h of chloranil addition, followed by a much slower decrease in A530 that was monitored for 220 h, as shown in Figure 3. At acidic pH, the rise in A530 occurred very slowly, and no decrease was observed over the 220-h reaction period. No effects of the buffer on reaction kinetics were noted, either by varying the buffer concentration or by substituting carbonate for phosphate. Molecular oxygen also had no effect on the reaction rate.

Data similar to that shown in Figure 3 (six pH values from 6.9 to 8.1) were used to estimate pseudo-first-order rate coefficients for hydrolysis of chloranil to TrCHQ (k1,obs) and for subsequent decomposition of TrCHQ to chloranilic acid and other products (k2,eff). Estimates of the rate coefficients as a function of hydroxide ion concentration are shown in Figure 4. The linear fit in each case supports a second-order reaction model involving hydroxide ion for each step in converting chloranil to chloranilic acid. Hancock et al. [11] proposed that each hydrolysis reaction occurs first by hydroxide addition followed by chloride elimination. Given the strong pH dependence of each step, it is likely that hydroxide addition is the rate-limiting step.
After 10 min of reaction, slightly less than 1 mol of peroxide was consumed per mole of chloranil added via a second pathway that was independent of pH. The reaction of a methylated derivative of chloranilic acid (or 7. Lines of best fit have slopes of 0.88 (r = 0.995) and 1.13 (r = 0.990), respectively. Vertical bars represent ±1 SD from triplicate samples at each chloranil concentration.

FIGURE 4. Pseudo-first-order rate coefficients as a function of hydroxide ion concentration for conversion of chloranil to TrCHQ (●) and for subsequent decomposition of TrCHQ (○). Vertical bars represent 95% confidence limits on the estimate for the rate coefficient at each hydroxide ion concentration.

The linear fits to the data shown in Figure 4 were used to estimate second-order rate constants at 23 °C of 6.2 × 10^-5 M^-1 h^-1 (r^2 = 0.980) for conversion of chloranil to TrCHQ and 1.4 × 10^-4 M^-1 h^-1 (r^2 = 0.990) for conversion of TrCHQ to chloranilic acid. From the rate constant for the first reaction, the half-life of chloranil at pH 7 is estimated to be only 1.1 h. If the apparent pH-independent decomposition reaction of TrCHQ is ignored, the half-life of this species at pH 7 is estimated to be about 21 d.

Reactivity with Hydrogen Peroxide. The stoichiometry of the reaction between chloranil and hydrogen peroxide is illustrated in Figure 5 for two different reaction times. After 10 min of reaction, slightly less than 1 mol of peroxide was consumed per mole of chloranil added (all of the added chloranil was consumed), while a stoichiometric ratio of slightly more than 1:1 was observed after 10 h of reaction. Chloride production was measured in a separate experiment in each case. The fit for k_{eff} did not pass through the origin, indicating that decomposition of TrCHQ occurred via a second pathway that was independent of pH. The existence of such a pathway was supported by the observation of a methylated derivative of chloranilic acid (or analogous species) in the ES spectra (Figure 2c) and the appearance of two overlapping peaks in HPLC analysis of chloranil mixtures containing methanol.

The linear fits to the data shown in Figure 4 were used to estimate second-order rate constants at 23 °C of 6.2 × 10^-5 M^-1 h^-1 (r^2 = 0.980) for conversion of chloranil to TrCHQ and 1.4 × 10^-4 M^-1 h^-1 (r^2 = 0.990) for conversion of TrCHQ to chloranilic acid. From the rate constant for the first reaction, the half-life of chloranil at pH 7 is estimated to be only 1.1 h. If the apparent pH-independent decomposition reaction of TrCHQ is ignored, the half-life of this species at pH 7 is estimated to be about 21 d.

Acute Toxicity Screening. The Microtox assay has been used as a screening tool to assess the potential acute aquatic toxicity of chemicals, environmental samples, and reaction products derived from waste treatment or remediation processes (18). Microtox EC_{50} values of PCP, chloranil, and a chloranil solution decomposed for 3.5 h all were in the range of 1–3 μM. For the chloranil solution incubated at pH 7 for 3.5 h, approximately 90% conversion to TrCHQ would have been expected; therefore, it appears that TrCHQ is about as toxic as chloranil. To the contrary, however, chloranil acid did not elicit a 50% attenuation in light
output in the Microtox assay at concentrations as high as 180 μM.

There was little change in toxicity when chloranil was decomposed with hydrogen peroxide. This finding supports the conclusion that products other than chloranilic acid are produced from the reaction between chloranil and peroxide.

**Conclusions**

*p*-Chloranil is a known toxic compound that has been observed as a significant product in processes used to oxidize pentachlorophenol. In aqueous solution, it undergoes two consecutive hydrolytic dechlorination reactions to yield chloranilic acid via the intermediate trichlorohydroxyquinone. Each hydrolysis reaction apparently occurs via displacement of chloride by hydroxide, so that the rate of hydrolysis is strongly pH dependent. Reaction rates are described by second-order kinetics, with estimated half-lives of chloranil and TrCHQ at pH 7 and 23 °C of 1.1 h and 21 d, respectively. Lesser chlorinated quinones, such as 2,6-dichloro-1,4-benzoquinone, have also been observed to undergo color changes in aqueous solution that occur with chloranil. Hydrolysis reactions involving alkyl halides have been well characterized (19), but the hydrolysis of chlorinated aromatics in environmental systems has not been well studied.

Hydrogen peroxide strongly accelerates the rate of decomposition of chloranil, but the low combined yield of TrCHQ and chloranilic acid from this reaction indicates that other products are formed. Peroxide-dependent decomposition pathways may be important in systems where peroxide is either used or produced, as in the peroxidase-catalyzed oxidation of PCP.

The final product of hydrolytic chloranil decomposition, chloranilic acid, appears to be significantly less toxic (as defined in the Microtox assay) than the parent compound. This finding may have implications for waste treatment or ultimate detoxification of PCP and its oxidation products. For example, it may be possible to expedite the remediation processes in which chloranil is formed from systems containing peroxide, and such reactions may influence the rates of decomposition reactions in the environment.

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**Literature Cited**


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