Transformation of Acetaminophen by Chlorination Produces the Toxicants 1,4-Benzoquinone and *N*-Acetyl-*p*-benzoquinone Imine

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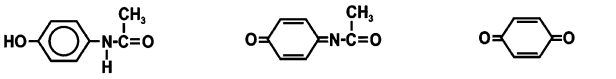
The reaction of the common pain reliever acetaminophen (paracetamol, 4-acetamidophenol) with hypochlorite was investigated over time under conditions that simulate wastewater disinfection. Initially, the reaction was studied in pure water at neutral pH (7.0), a range of reaction times (2−90 min), and a molar excess of hypochlorite (2−57 times) relative to the acetaminophen concentration. The reaction was monitored using reversed-phase liquid chromatography (LC) with ultraviolet absorbance, electrochemical, and mass spectrometric detection. At 1 μmol/L (150 ppb) and 10 μmol/L (1.5 ppm) levels, acetaminophen readily reacted to form at least 11 discernible products, all of which exhibited greater LC retention than the parent. Two of the products were unequivocally identified as the toxic compounds 1,4-benzoquinone and *N*-acetyl-*p*-benzoquinone imine (NAPQI), which is the toxicant associated with lethality in acetaminophen overdoses. With a hypochlorite dose of 57 μmol/L (4 ppm as Cl_2), 88% of the acetaminophen (10 μmol/L initial) was transformed in 1 h. The two quinoidal oxidation products 1,4-benzoquinone and NAPQI accounted for 25% and 1.5% of the initial acetaminophen concentration, respectively, at a 1 h reaction time. Other products that were identified included two ring chlorination products, chloro-4-acetamidophenol and dichloro-4-acetamidophenol, which combined were approximately 7% of the initial acetaminophen concentration at 1 h. The reaction was also studied in wastewater, where similar reactivity was noted. These results demonstrate that acetaminophen is likely to be transformed significantly during wastewater chlorination. The reactivity of the chlorine-transformation products was also studied with sulfite to simulate dechlorination, and 1,4-benzoquinone and NAPQI were completely reduced.

Introduction

Acetaminophen (I) is the most widely used over-the-counter analgesic in the U. S. with production of 3.6×10^9 g in 2002. It is a safe drug when consumed at therapeutic dosages, where the body metabolizes acetaminophen to labile sulfate and glucuronide conjugates for excretion (*1*). However, overdosage with acetaminophen can be fatal. At high doses, liver microsomes oxidize acetaminophen to *N*-acetyl-*p*-benzoquinone imine (NAPQI; II), a toxic metabolite that results in hepatic necrosis (*1*). NAPQI, however, is known to be fairly unstable and readily hydrolyzes to the toxicant 1,4-benzoquinone (III) in aqueous solution (*2*). The wide use and potentially nefarious chemistry exhibited by acetaminophen render it an important pharmaceutical compound to investigate in the environment. Acetaminophen has already been identified as one of the most frequently detected anthropogenic compounds in a survey of 139 streams in the U.S. by the United States Geological Survey (*3*). In their evaluation, acetaminophen was detected in as many as 24% of the samples with a median concentration of 0.73 nmol/L (0.11 ppb), but concentrations as high as 66 nmol/L (10 ppb) were also reported (*3*). Unlike many other more "traditional" persistent pollutants, little is known about the aquatic ecosystem effects of pharmaceutical compounds, which are less persistent but could pose a threat due to their continuous release into the environment (*4*).

CHART 1



Acetaminophen (I) | *N*-Acetyl-*p*-benzoquinone imine (NAPQI) (II) | 1,4-Benzoquinone (III)

Pharmaceutical compounds such as acetaminophen that are detected in the environment are subjected to both waste and drinking water treatment processes. While some researchers have investigated the effect of the individual treatment processes (e.g., biological digestion, chlorination) in reducing the concentrations of parent pharmaceutical compounds (*5*−*8*), little attention has been placed on their potential transformations during treatment. A study of activated sludge processing suggested that most hydrophilic pharmaceuticals such as acetaminophen exhibit negligible sorption onto sludge and incomplete biological transformation (*8*). Therefore, many pharmaceutical compounds are further subjected to chemical treatment processes. Chlorination is the most widely used chemical process for disinfecting wastewater and drinking water in the U. S. Chlorine is a strong, general oxidant that is capable of rapidly transforming pharmaceutical compounds with reactive functional groups such as the phenol found in the acetaminophen molecule. Understanding the chemical fate of acetaminophen and the toxicological nature of the chlorine-transformation products is an important first step to understanding its environmental significance. To date, limited studies of the transformation rates of parent pharmaceuticals such as acetaminophen and endocrine disruptors by chlorine have been reported (*9*−*11*). Identification of potential transformation products has been limited to an investigation of the sulfa drugs (*10*). Another significant chemical process that is often employed in wastewater treatment processing is dechlorination with a reducing agent like sulfite, although it is not as universally practiced as chlorination. Further reactions of the chlorine-transformation products of acetaminophen are possible during dechlorination and require consideration.

In this study, we characterize the acetaminophen chlorination products in both pure water and in wastewater under conditions encountered in wastewater treatment processes. The reactivity of acetaminophen and its chlorination products were further characterized by investigating dechlorination with sulfite. This study presents the most comprehensive

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evaluation of the reactivity of acetaminophen in the chemical processes used in wastewater treatment to date. All reactions and products were studied using reversed-phase liquid chromatography (LC) with ultraviolet absorbance (UV), electrochemical (EC), and mass spectrometric (MS) detection.

Experimental Section

Reagents. Acetaminophen (4-acetamidophenol; 98%) was purchased from Aldrich (Milwaukee, WI). Solutions of acetaminophen were prepared in pure water at different concentration levels, including 1, 10, and 337 μmol/L, and buffered with phosphate buffer, pH 7, to 1, 1, and 10 mmol/L total phosphate, respectively. The solutions also contained traces of methanol (<1% volume fraction), which aided in preparing solutions. Standards for 1,4-benzoquinone (98%) and NAPQI were purchased from Aldrich and Sigma (St. Louis, MO), respectively. Aqueous calibration solutions of 3 μmol/L 1,4-benzoquinone were freshly prepared and analyzed on days when chlorination experiments were performed. Due to the instability of NAPQI in water (*2*), a 117 μmol/L solution was prepared in acetonitrile and used for calibration.

For chlorination experiments, a 5% solution (> 5% as Cl) of reagent grade sodium hypochlorite (NaOCl) was obtained from Alfa Aesar (Ward Hill, MA) and diluted to approximately 5 mmol/L. The solution was standardized weekly using an iodometric titration, and the concentration was found to be stable over a period of months. At pH 7, the reactive chlorine species will be distributed between hypochlorite ion and hypochlorous acid based on the pK_a value of 7.5. In this paper we will refer to this equilibrium mixture as "hypochlorite". Subsamples of the acetaminophen solutions (1.0 mL) were chlorinated in an amber vial by adding an appropriate aliquot of the standardized NaOCl solution and vortex mixing for 10 s. Both the 1 and 10 μmol/L acetaminophen solutions were chlorinated to 57 μmol/L with hypochlorite, which is equivalent to 4 ppm as Cl_2, a representative disinfection dose used for wastewater treatment. A 337 μmol/L acetaminophen solution was chlorinated at 674 μmol/L OCl^- for mass spectrometric determinations. All chlorination experiments were performed at room temperature, 23 $\pm$ 1 °C.

For the dechlorination experiment, a solution of sodium sulfite (analytical reagent grade, Mallinckrodt, Paris, KY) was prepared in nitrogen-purged water. An aliquot was added to the 10 μmol/L acetaminophen chlorination mixture following 1 h of reaction time and vortex-mixed. The concentration of sulfite was made to be 84 μmol/L (1.5 times the initial hypochlorite dose). Dechlorination was allowed to proceed for 2 min.

Water (OmniSolv, EMD Chemicals, Gibbstown, NJ) and HPLC-grade methanol (Burdick and Jackson, Muskegon, MI) were used for the LC mobile phases. A 1 mol/L buffer solution was prepared from ammonium acetate and acetic acid (Mallinckrodt, Paris, KY) to be pH 5.8 and was diluted to prepare the mobile phases. Trifluoroacetic acid (TFA) was obtained from Fluka (Buchs, Switzerland) and used to prepare a mobile phase for MS with positive mode electrospray ionization, ESI^+.

Wastewater. Wastewater samples were collected from the Seneca Wastewater Treatment Plant, operated by the Washington Suburban Sanitary Commission in Germantown, MD. This plant utilizes an aerobic, biological activated sludge reactor. Chlorination is achieved with Cl_2(g) and a contact time of 1 h. Dechlorination is achieved with SO_2(g). The monthly average values during the time of sampling were ammonia-N 0.2 mg/L, organic-N 0.7 mg/L, and pH 7.5. Wastewater for laboratory chlorination was collected at a point downstream from biological digestion, clarification/sand filtration, and just prior to chlorination in the treatment process. The wastewater was transported to the lab and stored in a refrigerator at 4 °C. Before the wastewater was used in experiments, it was allowed to equilibrate to room temperature and centrifuged at 5000 rpm for 5 min to remove any suspended solids that were present. An acetaminophen solution was prepared to be 10 μmol/L in wastewater with 1 mmol/L pH 7 phosphate buffer. A subsample was chlorinated to 57 μmol/L as described for the pure water experiments and allowed to react for 1 h.

Chromatography. Samples were analyzed using two LC systems, both of which employed UV detection. The LC/UV/EC system consisted of two pumps (model 510, Waters, Milford, MA) and also employed an EC detector (model LC-4A, Bioanalytical Systems, West Lafayette, IN), while the LC/UV/MS system was an Agilent 1100 LC with an SL series mass selective detector (MSD; Palo Alto, CA) that used both ESI and atmospheric pressure chemical ionization (APCI) sources. Both systems utilized a Zorbax C-18 SB RP analytical column (Agilent) that was 3.0 mm × 250 mm and packed with 5 μm particles and a two-solvent isocratic mobile phase that consisted of 61% "A" and 39% "B." For LC/UV/EC, "A" was 50 mmol/L ammonium acetate/acetic acid buffer in water, pH 5.8. "B" was 99% (volume fraction) methanol, 1% (volume fraction) water, 10 mmol/L ammonium acetate/acetic acid buffer, pH 5.8. For LC/UV/MS, "A" was 10 mmol/L ammonium acetate/acetic acid buffer in water, pH 5.8, and "B" was the same as for LC/UV/EC when APCI was used and 99% (volume fraction) methanol, 1% (volume fraction) water, with TFA added to be 0.025% (volume fraction) when ESI^+ was used. The mobile phases were delivered at 0.4 mL/min. Injections of 50 μL were used for the 1 and 10 μmol/L acetaminophen solutions, while an injection of 40 μL was used for the 337 μmol/L solution. UV detection of acetaminophen and the reaction products was performed at 245 nm, which is the absorbance maximum for acetaminophen. Electrochemical detection on the LC/UV/EC system was performed at −0.1 V, which allowed for the selective detection of easily reducible oxidation products. MS detection was used with both ESI and APCI techniques. ESI^+ used N_2 drying gas flowing at 9.0 L/min and 350 °C, nebulizer pressure 0.24 MPa, fragmentor 75 V, and a capillary voltage of 3000 V. APCI was performed with negative polarity, N_2 drying gas flowing at 4.0 L/min and 350 °C, nebulizer pressure 0.41 MPa, vaporizer temperature of 300 °C, corona current 12 μA, fragmentor 125 V, and a capillary voltage of 3000 V. The mass spectrometer was scanned over the range from m/z 80 to m/z 380 to obtain spectra.

The linearity of the chromatographic responses was verified for acetaminophen, NAPQI, and 1,4-benzoquinone using at least three points, and R^2 values ranging from 0.99 to 1.00 were found. As a result of the remarkable linearity, single-point calibration factors were used and calculated as a ratio of the concentration to the peak area (assuming a zero intercept) using the standard solutions of acetaminophen (10 μmol/L), NAPQI (117 μmol/L), and 1,4-benzoquinone (3 μmol/L) described in the "reagents" section.

Results

Chlorination of Acetaminophen. In our acetaminophen/chlorination experiments, hypochlorite doses were selected to provide a significant excess of chlorine relative to the acetaminophen concentration, reflecting the large excess of chlorine in a typical wastewater treatment process. The reactions were monitored up to 90 min, a reaction time that is representative of the 1−2 h chlorine contact times used in wastewater treatment. Reactions were studied at pH 7 using buffered solutions. The chlorination of acetaminophen was initially evaluated using a liquid chromatographic separation and UV absorbance detection with the LC/UV/EC system. Figure 1 presents chromatograms obtained with UV detection for two different acetaminophen levels at a chlorination time of 10 min. The lower chromatogram reveals

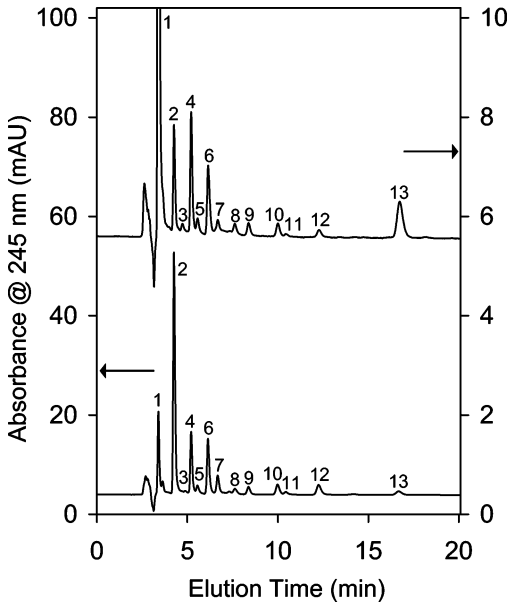

FIGURE 1. Separation of chlorination products for two acetaminophen concentration levels using the following conditions: reaction time 10 min, hypochlorite dose 57 μmol/L, 1 mmol/L pH 7 phosphate buffer, and temperature 23 $\pm$ 1 °C. Lower chromatogram: 10 μmol/L acetaminophen (left absorbance scale). Upper chromatogram: 1 μmol/L acetaminophen (right absorbance scale). Peaks numbered in order of elution. Peak identities: 1, monochloramine; 2, acetaminophen; all others, reaction products.

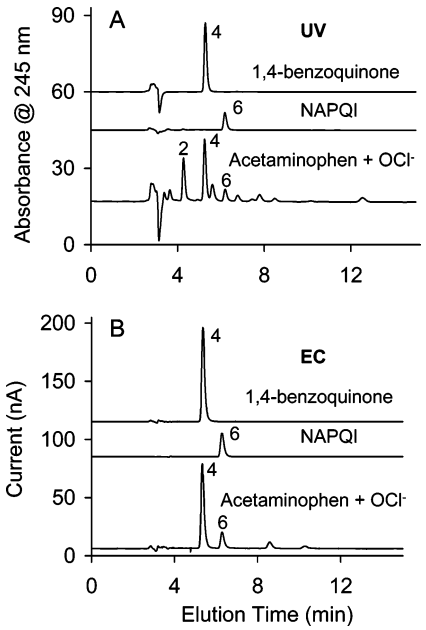

FIGURE 2. Separations of a chlorinated acetaminophen solution and standard solutions of 1,4-benzoquinone and NAPQI as determined with (A) UV and (B) EC detection. Acetaminophen concentration was 10 μmol/L with a hypochlorite dose of 57 μmol/L for a reaction time of 21 min. The concentration of 1,4-benzoquinone was 3.4 μmol/L, and the responses were divided by 2 to obtain a response similar to that observed in the sample. The concentration of NAPQI was 12 μmol/L, and the responses were divided by 10 for the same reason. Peak identities: 2, acetaminophen; 4, 1,4-benzoquinone; 6, NAPQI.

a separation of the products formed from reactions of a 10 μmol/L (1.5 ppm) acetaminophen solution with 57 μmol/L hypochlorite (left absorbance scale). The first critical observation is the preponderance of separated components in this chromatogram, with at least 13 peaks being discernible. Peak 2 is acetaminophen, and peak 1 corresponds to monochloramine formed from the reaction of the excess hypochlorite with the ammonium ion present in the mobile phase buffer. The remaining 11 peaks therefore are transformation products from the acetaminophen/hypochlorite reaction.

The upper chromatogram in Figure 1 represents chlorination of the 1 μmol/L acetaminophen level (0.15 ppm) with the same 57 μmol/L hypochlorite dose (right absorbance scale). This chromatogram is plotted on a scale $^1/_{10}$th that of the 10 μmol/L solution, so that the relative peak proportions may be compared. All of the products formed at the higher acetaminophen level are formed at the lower level. At both levels, peak 4 is the most prominent of the products at this absorbance wavelength. However, the relative proportions of the peaks are different in the two experiments. At the lower level, acetaminophen (peak 2) has reacted to a greater extent, as shown by the significantly lower relative peak height. Also, the peak heights of the product peaks are relatively greater at the lower level than at the higher level (in particular, peak 13), indicating more efficient conversion to products. The increased reactivity can be attributed to the greater molar excess of hypochlorite relative to acetaminophen (57×) at the lower level.

Chromatographic Identification of Reaction Products. To identify the products of the acetaminophen/hypochlorite reaction, we utilized LC with multiple detectors. Because hypochlorite is such a strong oxidant, we investigated whether the products of the reaction retained oxidizing ability. The electrochemical detector in the LC/UV/EC system evaluated the reducibility of the products and was used in tandem with the absorbance detector. The lowest traces in Figures 2A and 2B show chromatographic separations of the acetaminophen reaction products at 21 min obtained by UV and EC detection, respectively. The lowest UV chromatogram shown in Figure 2A is similar to the lower chromatogram in Figure 1, which is expected given the identical concentrations of acetaminophen and hypochlorite. The only difference in Figure 2A is the longer reaction time (21 min vs 10 min). The lowest EC chromatogram shown in Figure 2B reveals that four products are easily reducible. The number labels correspond to the same peaks as in Figure 1.

We investigated whether the acetaminophen/hypochlorite reaction might produce NAPQI using a chromatographic evaluation. A dilute aqueous solution of NAPQI was prepared from the calibrant solution in acetonitrile to be 12 μmol/L and analyzed immediately using the LC/UV/EC system; see middle traces in Figures 2A and 2B. Since NAPQI has a quinoid structure, it is very likely to be an oxidizing agent and therefore readily reducible. The chromatograms of NAPQI obtained by UV (Figure 2A) and EC (Figure 2B) indicate that it is detectable by both methods. More significantly, when the chromatograms for NAPQI are compared to the chlorinated acetaminophen solution in the lowest traces, it appears that peak 6 corresponds to NAPQI. On the basis of the equivalence of the retention times and two detection modes, NAPQI was tentatively identified as one of the oxidation products of acetaminophen. NAPQI is known to be relatively unstable in buffered aqueous solutions, where it is readily hydrolyzed to 1,4-benzoquinone (*2*). It is therefore likely that another of the detected products might be 1,4-benzoquinone. A solution of 1,4-benzoquinone was prepared and analyzed using the LC/UV/EC system, and the UV and EC chromatograms are shown as the uppermost traces in Figures 2A and 2B. As with NAPQI, 1,4-benzoquinone has a quinoid structure and is easily detectable using both detection modes. Also, the retention time for 1,4-benzoquinone in both the UV and the EC chromatograms corresponds with peak 4 in the chlorinated acetaminophen solution in the lowest traces, indicating the likely formation of this product.

We also investigated the potential formation of an N-chlorinated acetaminophen product using EC detection combined with iodide-postcolumn reaction chemistry (see method details in ref *12*). However, no chloramides were detected by this technique, in accord with prior observations that amide nitrogens, such as that contained within the acetaminophen structure, are not favorably N-chlorinated (*13*).

Mass Spectral Identification of Reaction Products. Mass spectrometric analysis using the LC/UV/MS system was employed to further elucidate the identities of the acetaminophen/hypochlorite reaction products using sufficiently high concentrations to provide full-scan spectra of the products. ESI was initially investigated because of its general utility for detecting many pharmaceutical compounds (*14*, *15*). Both positive and negative polarities were evaluated, but more definitive spectra were obtained using positive mode, ESI^+. A mass spectrum was obtained for the NAPQI product. The peak of highest intensity in the mass spectrum occurred at m/z 182.1, corresponding to the methanol adduct ion, $[M + H + \text{methanol}]^+$, where M for NAPQI is 149.0. The $[M + H]^+$ was less intense, but was present at m/z 150.1. These spectral characteristics were confirmed by MS analysis of the standard NAPQI solution.

Mass spectra were also obtained for some of the other products using ESI^+. The most prominent ion in the spectrum for peak 7 was m/z 186.1, but there was a second ion at m/z 188.1. The relative intensities of these two ions indicated the presence of one chlorine atom (as ^{35}Cl or ^{37}Cl) in the molecule. Given that the nominal molecular weight of acetaminophen is 151.1, an $[M + H]^+$ at m/z 186.1 corresponds to a monochlorinated acetaminophen molecule. Since we have evidence that no chloramide is formed in this reaction, we propose that this product derives from monochlorination of the aromatic ring of acetaminophen to form a chloro-4-acetamidophenol. The strongly activating phenol group probably directs the chlorine atom to the ortho position. As ESI is a soft ionization technique, there were no notable fragmentation ions in the mass spectrum to confirm the exact position of the chlorine on the aromatic ring.

A mass spectrum was also obtained for peak 12 in Figure 1. The most prominent ion was m/z 220.0, but ions at m/z 222.0 and m/z 224.1 were also notable. The 2 m/z unit spacing and relative intensities of these ions indicated the compound has two chlorine atoms, and the spectrum could therefore correspond to a dichlorinated acetaminophen. Prominent ions were also found at m/z 237.0 and m/z 239.0, which correspond to ions formed from addition of ammonium, $[M + NH_4]^+$, and sodium, $[M + Na]^+$, respectively. For reasons analogous to peak 7, peak 12 may be tentatively identified as a dichloro-4-acetamidophenol. Formation of mono- and dichloro-4-acetamidophenol was also noted in a study of the reaction of acetaminophen with hypochlorite for 48 h using LC/MS with a particle-beam interface (*16*).

We found it was not possible to obtain good spectra for 1,4-benzoquinone with ESI. However, a mass spectrum of 1,4-benzoquinone was obtained using APCI operated with negative polarity. There was only one prominent ion in the spectrum at m/z 108.1. Interestingly, the nominal molecular weight of 1,4-benzoquinone is 108.0, and the ion can be explained by either electron capture to form a radical anion or by a mass gain that was exactly balanced by a mass loss to make a negative ion. This same spectrum was obtained for a standard solution, indicating it is characteristic of 1,4-benzoquinone.

Some of the additional reaction products (peaks 3, 5, 7, 8, 9, 10, 11, and 13 in Figure 1) provided spectra that were not readily interpretable in either ESI or APCI modes. Several products had prominent ions with m/z of 300 or greater, indicating that coupling reactions such as dimerization of the acetaminophen had occurred. These products remain to be identified.

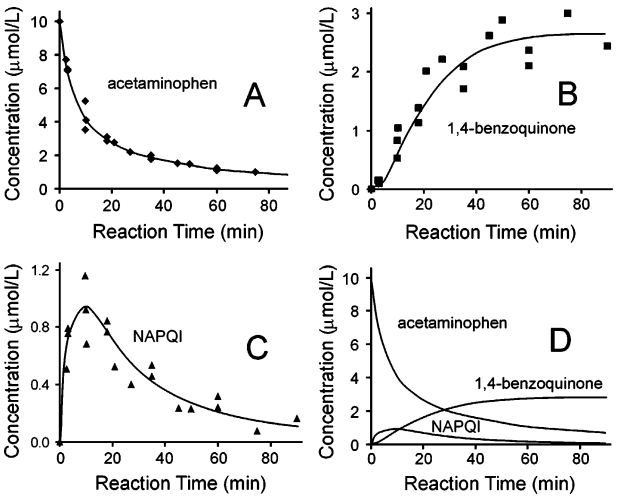

FIGURE 3. Concentrations of (A) acetaminophen and two of its reaction products (B) 1,4-benzoquinone and (C) NAPQI as a function of chlorine reaction time. Initial conditions: acetaminophen concentration 10 μmol/L; hypochlorite dose 57 μmol/L; 1 mmol/L phosphate buffer, pH 7; temperature 23 ± 1 °C. (D) Concentration trends of acetaminophen, NAPQI, and 1,4-benzoquinone as a function of time plotted on the same scale.

Time Course of Reaction. In addition to the identification of the products of the acetaminophen/hypochlorite reaction, it is also important to discern how the distribution of products varies with reaction time. An initial acetaminophen concentration $[A]_0$ of 10 μmol/L and a hypochlorite concentration of 57 μmol/L were used. The reaction mixture was chromatographically monitored over a period of 90 min by making successively later injections of the acetaminophen/hypochlorite solution. The time of injection was recorded as the reaction time. Upon injection, the chromatographic separation of acetaminophen from hypochlorite, combined with the large dilution factor, quenches further reaction. Peak areas of acetaminophen and its reaction products were then determined and converted to concentration units using response factors obtained from analysis of the standards. The concentrations for each of these compounds as a function of reaction time are shown in Figure 3, which includes all data from three replicate experiments. In the figure, the lines are drawn to help visualize trends and do not represent mathematical models. The loss of acetaminophen over time is shown in Figure 3A. At a reaction time of 60 min, the concentration of acetaminophen remaining is about 1.2 μmol/L (12% of $[A]_0$). The half-life of the reaction was graphically estimated to be 7.2 min under these conditions. The concentrations of the reaction products 1,4-benzoquinone and NAPQI as a function of time are shown in Figures 3B and 3C, respectively. We found that formation of NAPQI is favored initially, reaching a maximum concentration of about 1 μmol/L (10% of $[A]_0$) after 10 min, then decaying to about 0.15 μmol/L (1.5% of $[A]_0$) at 60 min). 1,4-Benzoquinone, however, increases over time, reaching a concentration of about 2.5 μmol/L (25% of $[A]_0$) at 60 min. Figure 3D plots the trend lines for acetaminophen, NAPQI, and 1,4-benzoquinone concentrations over time on the same scale to indicate their relative concentrations.

Pure compounds were not commercially available to quantify the formation of chloro-4-acetamidophenol and dichloro-4-acetamidophenol. Their concentrations were therefore estimated by assuming the molar absorptivity and response factor to be the same as acetaminophen. Consider, for example, the simpler system involving phenol and 2-chlorophenol, where molar absorptivity values of 2190 L

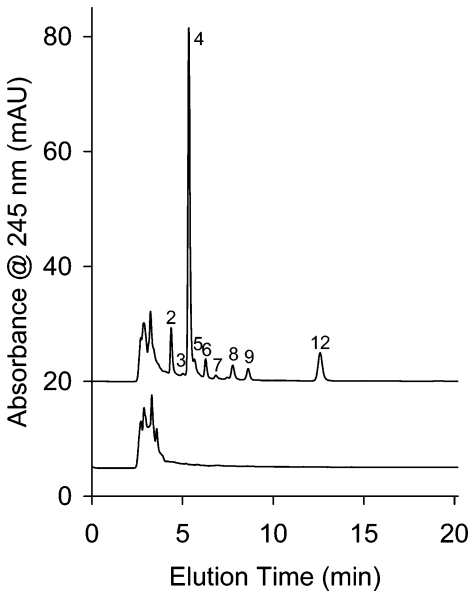

FIGURE 4. Separations of chlorinated wastewater after 1 h. Upper trace: chlorination of wastewater containing 10 μmol/L acetaminophen. Lower trace: chlorinated wastewater blank. Initial conditions: hypochlorite dose 57 μmol/L; 1 mmol/L phosphate buffer, pH 7; temperature 23 $\pm$ 1 °C. Peak identities: 1, monochloramine; 2, acetaminophen; 4, 1,4-benzoquinone; 6, NAPQI; 7, chloro-4-acetamidophenol; 12, dichloro-4-acetamidophenol.

cm^{-1} mol^{-1} and 2400 L cm^{-1} mol^{-1}, respectively, were found at the phenol absorbance maximum of 272 nm (NIST chemistry webbook, http://webbook.nist.gov/chemistry/). Since the addition of a chlorine atom to a phenol does not drastically change the absorptivity, we approximated the concentrations of chloro-4-acetamidophenol and dichloro-4-acetamidophenol using the molar response factor calculated for acetaminophen. After 60 min of reaction time, chloro-4-acetamidophenol and dichloro-4-acetamidophenol were approximated to be 0.3 μmol/L (3% of $[A]_0$) and 0.4 μmol/L (4% of $[A]_0$), respectively.

Dechlorination of Chlorinated Acetaminophen Mixture. The reactivity of the acetaminophen chlorination products toward dechlorination processes was evaluated using the common agent sulfite. After 1 h chlorination of the 10 μmol/L acetaminophen solution, sulfite was added in 1.5 times molar excess to the initial hypochlorite dose. After 2 min reaction time, the products were evaluated by LC. The NAPQI and 1,4-benzoquinone formed in the chlorination reaction were quantitatively reduced by the addition of sulfite, whereas the chloro- and dichloro-4-acetamidophenol products appeared to be unaffected. Additionally, the peak area of acetaminophen increased after dechlorination with sulfite.

Chlorination of Acetaminophen in Wastewater. The reactivity of acetaminophen with hypochlorite was studied in wastewater collected prior to chlorine disinfection at an operating treatment plant. A solution of 10 μmol/L acetaminophen was prepared in the wastewater and studied using the same chlorine dose, pH, and temperature as those used in the pure water experiments. The chromatographic separation of this reaction mixture after 1 h is shown as the upper trace in Figure 4. Also shown in the lower trace in this figure is a wastewater blank that had been chlorinated for 1 h. This wastewater clearly has components that absorb light at 245 nm as shown by the group of incompletely resolved peaks at the solvent front and the trailing absorbance from about 4 to 6 min. However, this blank does not prohibit the clear identification of acetaminophen (peak 2) and the chlorination products that are numbered in the upper chromatogram. All of the products that were identified in the pure water experiments, particularly 1,4-benzoquinone and NAPQI, are also formed in similar proportions in this wastewater.

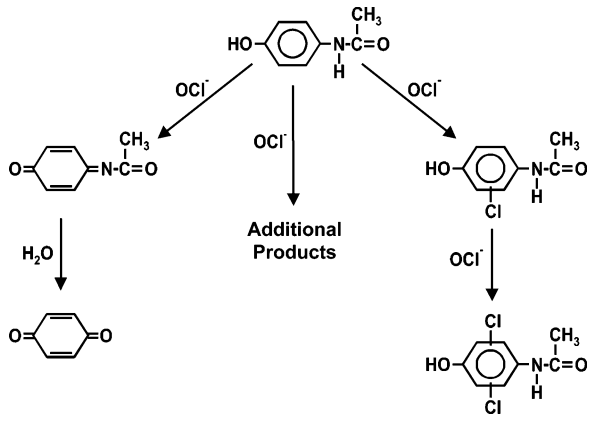

FIGURE 5. Reactions of acetaminophen with hypochlorite.

Discussion

Transformation of Acetaminophen by Hypochlorite. Acetaminophen exhibited significant reactivity with hypochlorite in pure water and in wastewater under conditions that closely simulate wastewater treatment: neutral pH, reaction times of approximately 1 h, and molar excess of hypochlorite. In all experiments, acetaminophen was transformed rapidly into multiple products. The reaction proceeded via a number of pathways: general oxidation to quinoidal products, ring chlorination, and possibly free-radical coupling. Because of the *p*-aminophenolic structure of acetaminophen, the formation of quinoidal oxidation products was enabled, leading to the production of NAPQI and 1,4-benzoquinone. Another reaction pathway observed was chlorination of the ring, leading to the products chloro-4-acetamidophenol and dichloro-4-acetamidophenol. Figure 5 summarizes the observed reactions of acetaminophen with hypochlorite. In addition, there was mass spectral evidence of the formation of several products with molecular weights over 300 in the acetaminophen/hypochlorite reaction in pure water. Such products are consistent with free-radical-coupling reactions via a semi-quinone imine or other radicals.

The reactivity of acetaminophen with hypochlorite was similar in pure water and this wastewater sample. However, this single wastewater sample cannot represent all wastewaters where such factors as the ammonia and amine content, pH, etc. are likely to modulate the rate of reaction and proportions of products. To understand the underlying chemistry, the reaction was characterized in detail using pure water at 1 h by determining the concentrations of the identified products relative to the initial acetaminophen concentration $[A]_0$. NAPQI and 1,4-benzoquinone were estimated to be 1.5% and 25% of $[A]_0$, respectively. Although the formation of NAPQI was initially favored (10% of $[A]_0$ at 10 min), 1,4-benzoquinone was a much more significant product as the reaction proceeded. When displayed on the same scale in Figure 3D, it suggests that NAPQI was the initial product of the oxidation reaction, which then quickly hydrolyzed to form 1,4-benzoquinone. The ring chlorination products choro-4-acetamidophenol and dichloro-4-acetamidophenol had a combined contribution estimated to be 7%. Therefore, the estimated concentrations of the known products accounted for 33% of the change in $[A]_0$ at 1 h. However, acetaminophen was not completely consumed in the reaction in 1 h, with 12% of $[A]_0$ remaining. From this semiquantitative evaluation, we have accounted for about 45% of $[A]_0$ at 1 h. We have been unable to conclusively identify products 3, 5, 8, 9, 10, 11, and 13, so determining their relative contributions is not possible. In addition to these UV-

absorbing products, there are also likely to be transformation products formed that have lost the chromophoric structure present in the acetaminophen molecule by such reactions as oxidative opening of the aromatic ring. This has been demonstrated for the reaction of paracetamol (acetaminophen) with ozone, which forms many small molecule products that have lost the aromaticity found in the parent (*17*).

The reactivity of acetaminophen with chlorine observed in our experiments is in general accord with a previous study that predicted the reaction rate of acetaminophen with hypochlorite would be sufficiently fast to be significant in many chlorine disinfection systems (*9*). However, the half-life value determined in this study, 7.2 min, is shorter than would be projected from the estimate provided in the previous investigation (*9*). Assuming a second-order kinetic model and accounting for the differences in reactant concentrations in our experiment from Table 2 of ref *9*, the half-life would be expected to be approximately 26 min. We believe that the use of thiosulfate to reductively quench chlorine in the previous investigation (*9*) concomitantly reduced NAPQI and 1,4-benzoquinone to acetaminophen and 1,4-hydroquinone, respectively. Acetaminophen produced by reduction would be measured as unreacted acetaminophen in their chromatographic separation, leading to an anomalously low determined reaction rate. This is supported by the results from our dechlorination experiment, where the acetaminophen peak area was greater after addition of sulfite and the NAPQI product was completely reduced.

Environmental Implications. In the pure water and wastewater experiments, most of the acetaminophen was transformed into new products via chlorination. It is therefore prudent to consider the toxicity and reactivity of these products. When LD_{50} toxicity values (intraperitoneal injections in mouse) are compared, acetaminophen has a value of 500 mg/kg (*2*), while 1,4-benzoquinone and NAPQI have significantly lower LD_{50} values of 8.5 mg/kg (*18*) and 20 mg/kg (*2*), respectively. 1,4-Benzoquinone and NAPQI are therefore approximately 58 and 25 times more toxic than acetaminophen, respectively. In humans, NAPQI is of particular concern due to its hepatotoxicity in acetaminophen overdoses. 1,4-Benzoquinone is a benzene metabolite implicated with genotoxic and mutagenic effects (*19*). In addition to their toxicity, both NAPQI and 1,4-benzoquinone showed significant reactivity in our experiments. In our LC analysis of buffered aqueous solutions, NAPQI began to produce significant amounts of 1,4-benzoquinone over the period of an hour. Neutrally buffered aqueous 1,4-benzoquinone solutions also demonstrated instability over the period of days, possibly via nucleophilic addition of water to form 2-hydroxyhydroquinone (*20*). In wastewater treatment operations that employ dechlorination with sulfite, it is likely that NAPQI will be reduced to acetaminophen and 1,4-benzoquinone will be converted into 1,4-hydroquinone. Thus, due to these multiple reaction modes, NAPQI and 1,4-benzoquinone are unlikely to persist in the environment. However, their toxicity and mutagenicity are a concern for the immediate environment continuously receiving chlorine-treated wastewater, particularly near plants that do not employ dechlorination. Unfortunately, no toxicity data or reactivity data are available for the chloro- and dichloro-4-acetamidophenol products, although they might be expected to have similar aqueous stability to acetaminophen. In our LC evaluations, unbuffered and neutrally buffered aqueous acetaminophen solutions demonstrated good stability for at least several weeks. Although formed in smaller amounts, chloro- and dichloro-4-acetamidophenol may also have environmental impact due to their potential persistence and increased hydrophobicity relative to acetaminophen.

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