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Effects of Chlorination on the Persistence of Pharmaceuticals in the Environment

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In the past decade, the identification of pharmaceuticals in surface (Jones et al. 2001; Ternes 1998), ground (Sacher et al. 2001) and drinking (Heberer 2002) waters has attracted the attention of both the scientific and lay communities. Although the concentrations of these compounds are very small (typically less than 1 μg/L), their presence is a reminder that many people's drinking water was once another community's wastewater. To date, the majority of the studies looking for pharmaceutical compounds have been conducted in Europe. Due to the differences in drinking and wastewater treatment technologies, as well as the dosage level and types of pharmaceuticals administered, it is difficult to estimate the concentrations of pharmaceuticals present in the waters of the United States based on European data. The United States Geological Survey (USGS) has recently conducted the first nationwide reconnaissance of pharmaceuticals (both human and veterinary) and other wastewater components in the United States (Kolpin et al. 2002). They found at least one of their target non-prescription drugs in approximately 80 % of the 139 streams sampled. At least one prescription drug was found in over 30 % of the streams and at least one antibiotic was found in over 50 % of the sampled locations. While the sample locations were biased towards areas that were expected to yield detections (such as downstream from urban centers and areas if dense livestock populations), this study demonstrates the pervasiveness of pharmaceuticals in the aquatic environment of the United States.

Due to the low concentrations of pharmaceuticals in the environment, most of the mass spectrometry methods that have been developed only measure known target compounds. These methods only monitor selected ions, which helps to improve detection limits. However, this improvement comes at a price. Since only specified ions are monitored, in studies where the influent and effluent of waste and drinking water treatment plants were analyzed, only removal efficiencies can be reported (Ternes 1998; Ternes et al. 2002; Zwiener and Frimmel 2000). The ultimate fate of these compounds, that is whether they were degraded into something harmless, or potentially transformed into something more toxic, cannot be determined.

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In the United States, chlorine is commonly used to disinfect sewage, as well as drinking water (USEPA 1999a). Although this procedure removes pathogens, the chlorine can also react with compounds in the water. As past research on disinfection byproducts in water has shown, this addition of chlorine may increase the toxicity of the compounds (Clark et al. 2001; Nieuwenhuijsen et al. 2000). Thus, if the removal of pharmaceuticals in waste and drinking water treatment plants is due to the chlorination of the compounds, the potential negative human health impacts may be increased rather than decreased. Mass spectrometry is useful in chlorination experiments, because it can easily detect the formation of new degradation products which contain chlorine atoms.

The purpose of this work was to determine via benchtop experiments the fate of pharmaceuticals during chlorination. This information can be used to focus occurrence studies on the analytes that have a higher probability of persisting through treatment (and thus being found in the environment), as well as the disinfection/ degradation byproducts that might be unknowingly produced.

MATERIALS AND METHODS

Commercially available pharmaceutical active ingredients were purchased from Sigma (St. Louis, MO). Table 1 lists the compounds investigated in this project, as well as the molecular weights and Chemical Abstracts Service (CAS) number. The analytes were chosen based on use in the United States, inclusion in the USGS reconnaissance study (Kolpin et al. 2002), and commercial availability. For each compound considered, two solutions, each with a concentration of 50 mg/ L, were made in Milli-Q water. Samples were made and stored in amber glass bottles to minimize photo-degradation. If the sample was not readily water soluble, such as the case with aspirin and trimethoprim, the sample was first made up at a concentration of 100 mg/ mL in methanol (Optima grade, Fisher Scientific, Pittsburgh, PA) and an aliquot of this sample was diluted to the desired final concentration with Milli-Q water. To one of the two solutions, sodium hypochlorite (Purified grade, Fisher Scientific) was added as a chlorinating agent. The concentration of chlorine in the sodium hypochlorite was determined using Standard Method 4500-Cl F: DPD Ferrous Titrometric Method (Eaton et al. 1995). The final concentration in the analyzed solutions of 28.75 mg Cl_2/ L was much higher than the 0.5- 2 mg/ L used in drinking water treatment (USEPA 1999b), but close to the 5- 20 mg/ L used in wastewater treatment (USEPA 1999c). Due to the relatively high concentration of the pharmaceutical solution, the additional hypochlorite was needed to see the effect of chlorination.

The non-chlorinated solutions were analyzed using liquid chromatography/mass spectrometry immediately following preparation, to obtain reference spectra. The samples were then allowed to sit on the benchtop in amber glass at room temperature for 48 hours, to allow the reaction to proceed and residual chlorine to dissipate. At this time, the chlorinated and non-chlorinated samples were both

Table 1. Compilation of pharmaceutical mass spectral ions observed in non-chlorinated and chlorinated solutions.

Compound	CAS	MW		RT (min)	Ions observed	Type[c]	USGS Freq[d]
Acetaminophen	103-90-2	151.2	No	6.62	151, 109	C	23.8
			Cl	6.77	151, 109		
				7.49	177[b], 109		
				8.01	185[a], 143[a], 109		
Aspirin	50-78-2	180.2	No	4.80	138, 120, 92	U	n/a
			Cl	4.96	138, 120, 92		
Amoxicillin	26787-78-0	365.4	No	4.98	313, 169, 122, 120, 107, 100	A	n/a
			Cl	6.14	313, 169, 119, 97		
Aspartame	22869-47-0	294.3	No	6.48	162, 131, 91	U	n/a
			Cl	6.64	162, 131, 91		
Caffeine	58-08-2	194.2	No	6.40	194, 109, 82	U	61.9
			Cl	6.48	194, 109, 82		
Cephalexin	15686-71-2	347.4	No	4.69	169, 147, 106	A	n/a
				6.11	169, 147, 106		
			Cl	4.70	169, 149, 119, 105, 82		
Cimetidine	51481-61-9	252.3	No	6.79	116, 109, 95, 94, 82	A	9.5
			Cl	5.71	112, 109, 95, 94, 83		
Cotinine	486-56-6	176.2	No	6.48	176, 98	U	38.1
			Cl	6.56	176, 98		
Diltiazem	33286-22-5	451.0	No	20.47	161, 150, 137, 121, 109	A	13.1
			Cl	6.89	135		
				7.55	135		
1,7-Dimethyl-xanthine	611-59-6	180.2	No	6.02	180, 123, 95	U	28.6
			Cl	6.02	180, 123, 95		
Gemfibrozil	25812-30-3	250.3	No	15.76	122, 107, 91	C	3.6
			Cl	20.80	156[a], 129, 121, 91		
6α-methyl-17α-hydroxy progesterone acetate	71-58-9	386.5	No	25.44	301, 283, 243	U	n/a
			Cl	25.36	301, 283, 243		
Trimethoprim	738-70-5	290.3	No	8.32	290, 259, 243, 123	A	27.4
			Cl	8.50	196, 181, 84		
Warfarin	129-06-6	330.3	No	7.44	145, 131, 103	A	0
			Cl	6.48	207, 169, 147, 121,		
				25.76	109, 223,155, 134, 121, 103, 91		

[a]Compound is singly chlorinated.
[b]Compound is doubly chlorinated.
[c]C= Chlorinated , U= Unchanged, A= Ambiguous -contains unknown or unidentified products.
[d]Frequency of occurrence in USGS pharmaceutical study (Kolpin et al 2002)

reanalyzed. Both the mass chromatograms and spectra of the non-chlorinated 48-hour samples were compared to the initial chromatograms and reference spectra to see if there were any changes over the hold time not associated with chlorination. Likewise, the chlorinated and non-chlorinated 48-hour mass chromatograms and spectra were compared to determine the effects of chlorination on the compounds.

Sets of samples (each set consisting of one non-chlorinated and one chlorinated sample) were prepared and analyzed using liquid chromatography/ mass spectrometry (LC/MS) on a Hewlett-Packard (now Agilent; Palo Alto, CA) particle beam system (PB), which consisted of an HP1090 liquid chromatograph coupled to an HP5989A MS Engine, through an HP59980 particle beam interface. A 25 µL aliquot of a sample was injected onto a 150 × 4.6 mm Inertsil ODS-2 liquid chromatography column (SGE, Austin, TX). The elution gradient began with a one-minute hold at 70 % 10 mM ammonium acetate (Fisher Scientific) and 30 % acetonitrile (Optima grade, Fisher Scientific). The mixture progressed to 100 % acetonitrile in 30 minutes at a flow rate of 0.4 mL/ minute. In the PB, helium was the nebulizing gas, set at 38 psi. The desolvation temperature was 55°C; the source temperature was 250°C. The scan range was set to *m/z* 80- 500.

The particle beam interface system was advantageous for this research because, unlike the other common mass spectrometry interfaces (i.e. electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI)), which produce just the molecular ions (M^{+*}) or protonated molecules ($(M + H)^{+}$), the mass spectra are comprised of multiple fragments, similar to the electron ionization (EI) spectra. The additional peaks can facilitate structure elucidation for those compounds that show spectral changes. However, the particle beam generates neither linear, nor stable calibration curves (Bellar et al. 1990; Behymer et al. 1990) and the sensitivity of the system was poor (hence the 50 mg/ L samples), so only qualitative results were obtained for this project.

RESULTS AND DISCUSSION.

The pharmaceutical compounds fell into three distinct groups: those that were unchanged, those that were chlorinated, and those that exhibited changes in their mass chromatograms and spectra due to the disinfection, but were not directly chlorinated. The most straightforward group of compounds to explain are those that did not demonstrate a change due to chlorination. Of the compounds investigated, six– cotinine (a nicotine metabolite), aspartame, caffeine, 1,7-dimethylxantine (a caffeine metabolite), aspirin, and 6α-methyl-17α-hydroxy progesterone acetate (a birth control agent)– showed no discernable differences between the PB mass chromatograms and spectra of the non-chlorinated and chlorinated samples. Retention times of the observed mass chromatographic peaks, as well as the observed mass spectral ions are listed in Table 1.

Of the fourteen target analytes, only two showed definite signs of chlorination: gemfibrozil and acetaminophen. The chlorinated gemfibrozil mass chromatogram only had one peak, but it was shifted out by five minutes, as compared to the mass chromatogram generated by non-chlorinated gemfibrozil. The predominant ion in the non-chlorinated sample was *m/z* 122. In the chlorinated sample, the mass spectrum shifted by 34 u ($+^{35}Cl$-^{1}H), so the principal ion was at *m/z* 156, and the distinctive ^{37}Cl isotope was present at *m/z* 158. It should be noted that gemfibrozil was the only compound analyzed that had some stability problems in the 48-hour hold time between experiments. The 48-hour non-chlorinated sample had an identical mass spectrum when compared to that taken immediately after sample preparation, but the abundance of the peak in the mass chromatogram was less than one-fifth of the fresh sample.

The acetaminophen chlorination was slightly more complex. While the addition of one chlorine atom to the acetaminophen molecule was the primary product of the reaction, some of the acetaminophen was doubly chlorinated, and some remained unreacted, see Figure 1.

The last group of six pharmaceuticals consisted of those that were changed by chlorination, but did not appear to become chlorinated, and are the most difficult to interpret. These compounds fell into two sub-categories. Amoxicillin, cephalexin and cimetidine fall into the first group– those that had one peak in the chlorinated mass chromatogram, slightly shifted (< 2 minutes) from the non-chlorinated sample. The mass spectra for these samples have some ions in common between the chlorinated and non-chlorinated versions, but, in all three cases, there were predominant ions in the non-chlorinated sample that were missing in the chlorinated sample. The last three compounds, trimethoprim, diltiazem and warfarin all exhibited multiple peaks in the chlorinated mass chromatogram, strongly shifted (3-20 minutes) away from the non-chlorinated solution's retention time. The mass spectra of these products were also different than the original compounds (see Table 1).

The utility of these model solutions can be seen when the results are compared to those obtained by Kolpin et al. (2002) in the USGS' national reconnaissance study. Nine of the fourteen analytes described in this paper were also target compounds in the USGS study. The frequencies of detection for these compounds in the USGS study are listed in Table 1. The compounds that remained mostly unaffected by chlorination– cotinine, caffeine, 1,7-dimethylxanthine, and trimethoprim– were found in greater than 25 % of the samples (caffeine was found in over 60 % of the samples). The pharmaceuticals that were either chlorinated or degraded during treatment– cimetidine, diltiazem, gemfibrozil and warfarin– were found in less than 14 % of the collected samples (the last two compounds were found in less than 4 % of the samples). The only pharmaceutical that did not fit this pattern was acetaminophen. While it showed definite signs of chlorination, acetaminophen was found in 23.8 % of the samples

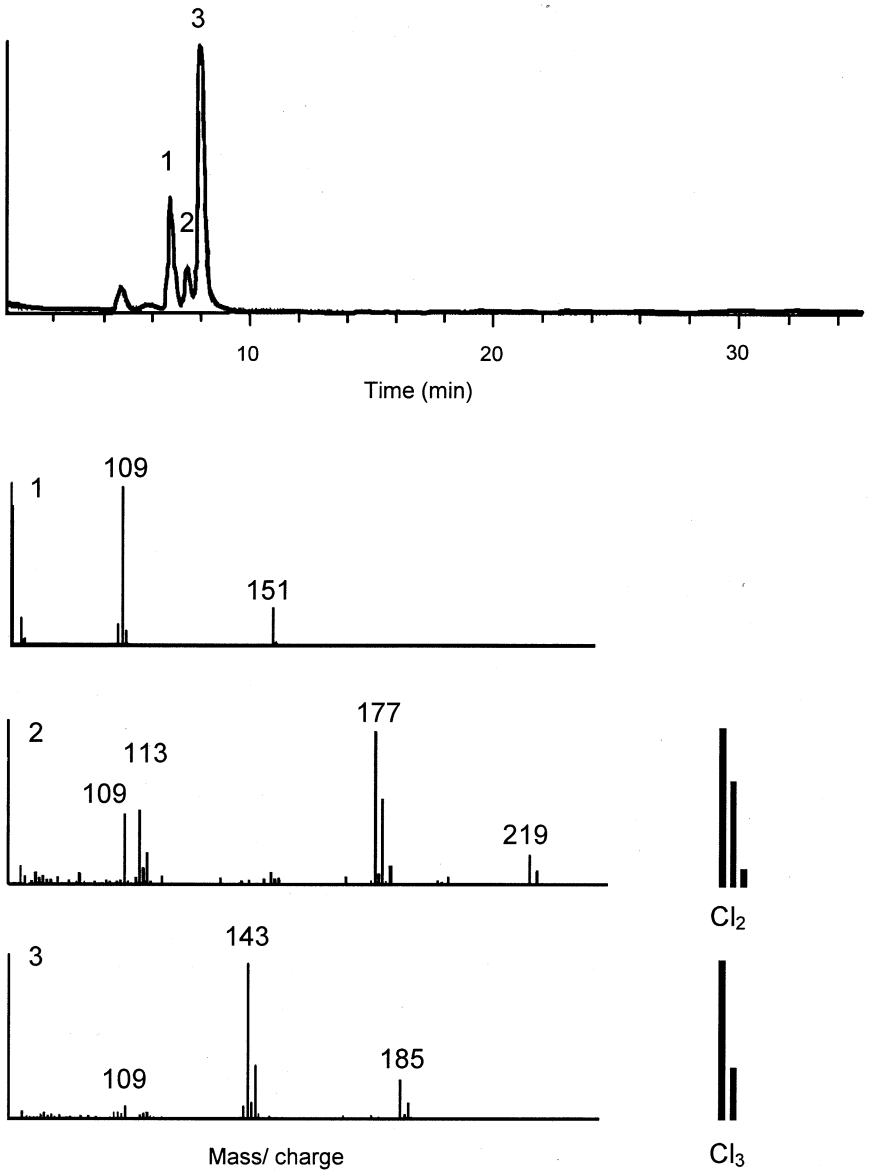

Figure 1. Experimental particle beam mass spectrometry results from acetaminophen chlorination. The chlorinated mass chromatogram is presented at the top, followed by the mass spectra of the three products: (1) unreacted acetaminophen, (2) doubly chlorinated acetaminophen and (3) singly chlorinated acetaminophen. The peak at approximately 4.5 minutes is an artifact of chlorination, present in every sample. For reference purposes, the expected isotope ratio patterns for a doubly and a singly chlorinated compound are presented beside mass spectra 2 and 3.

analyzed by USGS. However, some of the pharmaceutical was not chlorinated in the model solution, and it is very commonly used, so its persistence is not entirely unexpected. This research shows that model disinfection solutions are tools that can be used to predict the survival of pharmaceuticals and other contaminants through waste and drinking water treatment. The use of these studies as pre-screening tools would allow potential analyte lists to be truncated to include only those compounds that have the best chance of detection in environmental samples. It would also help to determine what other important disinfection byproducts are common and should be monitored to determine the true importance of a given pharmaceutical on the environment.

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