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Development of a field-practical assay for water-solvated chlorophenols

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Abstract

A field-practical assay for chlorophenols (CPs) was developed and the accuracy, specificity and sensitivity were defined. The assay was capable of detecting these ubiquitous toxic compounds in water and has the potential for being used as a rapid, inexpensive screening test for potable water. The solvated target molecules were labeled with the fluorescent tag, 5-dimethylaminonaphthalene-1-sulfonyl chloride, dansyl chloride (DsCl). Extraction of the fluorescent complex allowed for the removal of co-contaminants. Detection of the dansylated CPs was based on the visualization of induced bluish-yellow fluorescence. The accuracy, specificity and sensitivity of the assay were determined by: (i) testing diverse organic and inorganic contaminants (listed in the 1992 US Primary Drinking Water Standards), (ii) testing aqueous CP solutions of known concentration, and iii) testing potable water samples collected from six Texas cities.

1. Introduction

The environmental impact of the chlorophenols (CPs) is of great concern to the scientific community. The CPs have become ubiquitous environmental contaminants because of their widespread use as wood-preserving agents, biocides, and in the manufacturing of plastics [1,2]. They demonstrate notable toxicity and environmental stability, making them a threat to human health [3,4]. Net daily exposures for people not specifically exposed to pentachlorophenol (C_5P) range from 5 μg in Nigeria to 37 μg in the Netherlands, with much greater exposure being found in those who work with, or near a source of CPs [5].

It is well documented that the CPs are lipophilic and are readily capable of interfering with cellular respiration [6]. The toxicity usually parallels the degree of

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chlorination, making C_5P several orders of magnitude more toxic than the monochlorophenols [7]. Cell death can then be followed by organ disfunction and/or death. The CPs are potent hepatotoxins and are known to compromise the human immune system [8,9]. The possible carcinogenicity of the CPs is highly controversial [10], but it has been reported that exposure can be linked to statistically significant increases in soft-tissue sarcomas and non-Hodgkin's lymphomas [11].

The toxicity and environmental prevalence of water-solvated CPs make their rapid detection very desirable. The classical approach to the detection of CPs has been instrumental in nature, with an emphasis on high performance liquid chromatography (HPLC) [12] and gas chromatography (GC) [13]. More current methods include improved HPLC [14] and sophisticated hybrid techniques such as gas chromatography/mass spectrometry (GC/MS) [15]. All of these approaches are expensive, require significant technical expertise, and are confined to a laboratory setting.

During the 1970s, it became apparent that the labeling of the CPs with a fluorescent moiety could improve the sensitivity, detection limits, and resolution of HPLC analysis [16,17]. The same was true for GC [18]. During the 1980s, it was shown that there are unique and dynamic interactions between CPs and certain inorganic sorbent materials. Alum, which is aluminum-based, tends to bind CPs very tightly and with great selectivity [19].

Based on these findings, the major objective of this study was to construct a simple testing device (i.e., a packed glass minicolumn) that could be used to resolve rapidly, selectively immobilize, and visually detect the CPs from samples of contaminated potable water.

2. Materials and methods

2.1. Chemicals and materials

The CPs, other than the tetrachlorophenols, were purchased from Sigma (St. Louis, MO). The tetrachlorophenols were synthesized from their anisole analogs. GC/MS (Hewlett Packard, Palo Alto, CA) was used to confirm the identity and verify the purity of the synthesized tetrachlorophenols [7]. DsCl was purchased from Sigma (St. Louis, MO). The herbicides – 2,4-dichlorophenoxyacetic acid (2,4-D) and methoxychlor were purchased from Chem Service (West Chester, PA). The 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was purchased from PolyScience (Niles, IL). NovaSil™ was obtained from Engelhard Corporation (Cleveland, OH). All other chemicals were used without further purification. Ultrapure water was generated in this laboratory following distillation, using a Continental water system (Houston, TX) that consisted of mixed bed ion exchange resins and final purification via reverse osmosis. Aqueous solutions of 10 parts per thousand (ppth), 1 ppth, 100 ppm, 10 ppm, 1 ppm, 100 ppb, 1 ppb, and 100 parts per trillion (ppt) were prepared for each CP by successive dilution when their solubility allowed. An aqueous solution of 500 ppm diethyl amine (DEA) was also made. Aqueous 1 *N* sodium carbonate and 1 *N* sodium hydroxide were prepared with ultrapure water. A solution of 1 mg/ml DsCl was made

with spectral grade glass-distilled anhydrous acetone and stored in a refrigerated desiccator (4 °C).

2.2. Minicolumn construction

The minicolumn was designed to screen potable water supplies for CP contamination quickly, simply, and at a fraction of the cost of standard methods. A borosilicate glass blood dispensing pipette was used to house the packing materials. Regular glass pipettes were fluorescent in the presence of longwave UV and masked the fluorescence of the target CP derivatives. Borosilicate glass was not fluorescent and offered the advantage of being less fragile than regular glass. Laboratory-grade sand was used as a spacer between sorbent layers in the design of the minicolumn. The solvents that were used in this assay and DsCl did not interact with the sand. Prior to use, the sand was rinsed with anhydrous acetone and allowed to dry. Once the sand was packed into the column, it did an excellent job of holding the preparative layers and the reactive interface (sand/sorbent) in place. The volume of sand did not affect the results as long as there was enough sand to separate the reactive layers. A non-reactive polyethylene plug was used to support the layers of the minicolumn.

DsCl binds with centers of high electron density and readily reacts with the CPs to form a highly fluorescent complex (Fig. 1). This complex can be extracted, eluted, and immobilized at a reactive interface of sand/neutral alumina in the minicolumn. Neutral alumina was chosen due to its ability to strongly bind dansylated CPs (which have no affinity for sand). Also, there was minimal interaction between anhydrous acetone, methylene chloride, water, and the alumina. The neutral alumina was packed

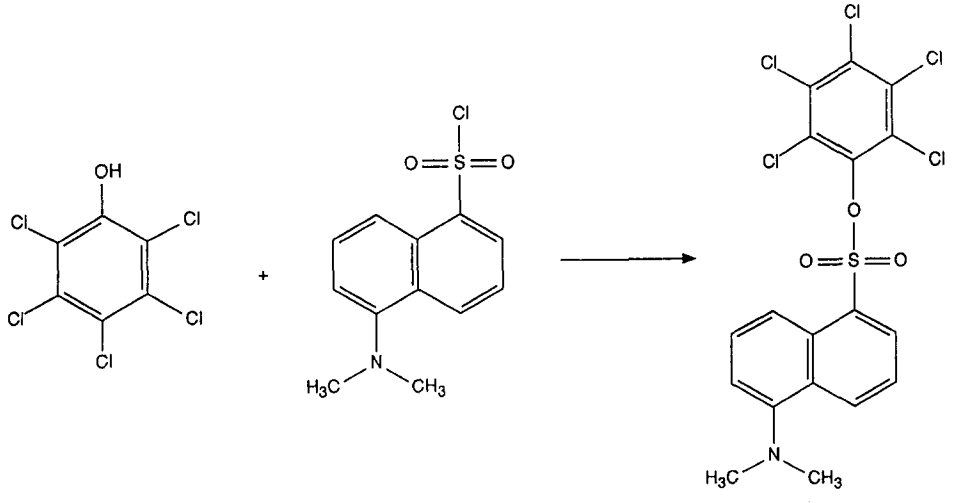

Fig. 1. Reaction of pentachlorophenol with the fluorescent label, 5-dimethylaminonaphthalene-1-sulfonyl chloride, DsCl. The reaction is representative of the reaction with all chlorophenols.

in a 2 mm layer. The activity of the alumina (water content) had an effect on its ability to bind the fluorescent complex. Activities less than 3.0 (6% water) were avoided to prevent a loss of selectivity and of intensity and color of induced fluorescence.

Since DsCl can react with amines which may be present in potable water and interfere with the test, it was necessary to add a thin layer of phyllosilicate clay (NovaSil™) to the minicolumn. The sorbent removed the dansylated amine from the eluate, but allowed the dansylated target molecules (i.e., CPs) to pass. Anhydrous acetone, methylene chloride, and water did not interfere with the passage of dansylated CPs to the reactive interface. As with the sand, the sorbent was rinsed with anhydrous acetone and allowed to dry before being used. A 1 mm preparative layer was used (Fig. 2).

2.3. Assay procedure

The procedure used in labeling the aqueous CPs was a modification of the procedure reported by Lawrence et al. [12]. A 20 µl aliquot of the water was mixed

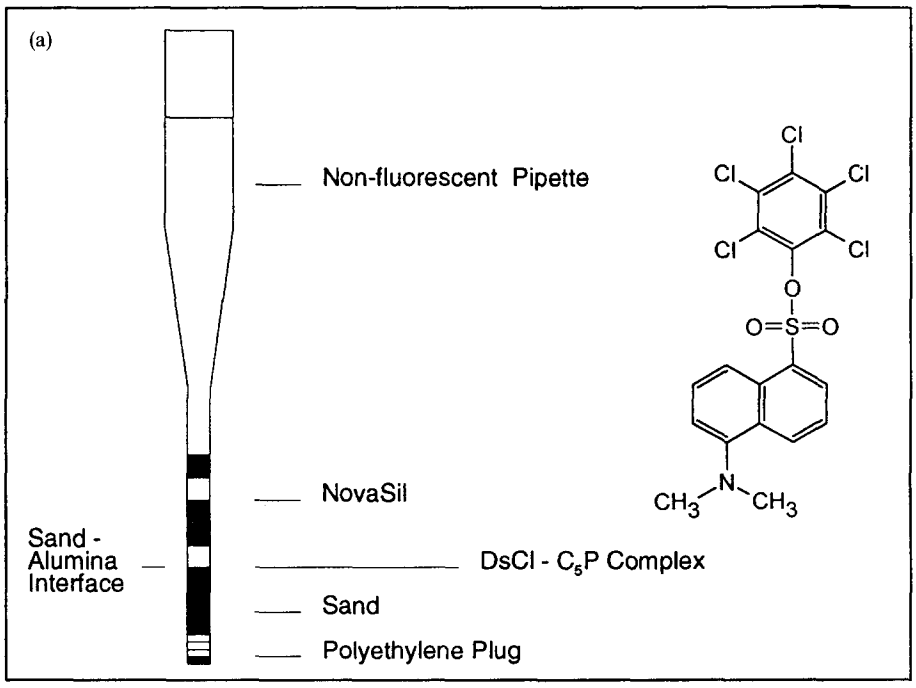

Fig. 2. (a) Diagram of minicolumn assay for water-solvated chlorophenols. The dansyl chloride-tagged chlorophenol is bound at an alumina–sand interface and detected by induced fluorescence. (b) Glass minicolumn assay units for the detection of water-solvated chlorophenols. (c) Induced fluorescence and detection of varying concentrations of aqueous pentachlorophenol. The fluorescence corresponds to 0, 0.1, 1 ppb, 1 and 1000 ppm aqueous pentachlorophenol. While there is a relationship between concentration of pentachlorophenol and degree of fluorescence, this assay is not quantitative in nature.

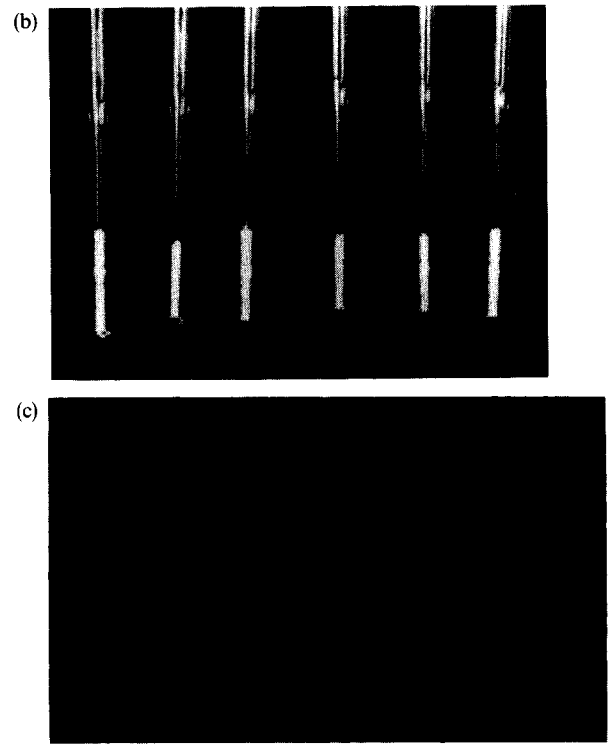

Fig. 2. Continued.

with a tenfold excess of DsCl solution, allowing for an excess of derivatizing agent. 20 μl of 1 *N* sodium carbonate was added to promote the formation of the chlorophenate anion which was essential to the formation of the complex. Addition of 1 ml 1 *N* sodium hydroxide served two purposes: it solvated unreacted DsCl and other water-soluble impurities, while destroying unstable complexation products such as dansylated carboxylic acids. 500 μl of methylene chloride was added to provide ample organic phase for extraction. The mixture was agitated by hand. A suction bulb was used to force the solution through the minicolumn. 1 ml of anhydrous acetone was forced through the column to wash all remaining products through the minicolumn. A hand-held UV light was used to induce fluorescence at the reactive interface when CPs were present in the water sample. If bright fluorescence was detected, the test was considered to be positive.

2.4. Testing

The approximate limit of detection (LOD) and specificity of the assay was established for seventeen CP isomers commonly encountered in aqueous solution. The approximate LOD was determined. For each CP, three aliquots at a concentration 10 times greater than the approximate LOD, three aliquots at a concentration equal to

the approximate LOD, and three aliquots at a concentration of 10% of the approximate LOD were assayed. The CPs required approximate LODs because maximum contaminant levels (MCLs) have yet to be established for all of these chemicals. Five samples of aqueous 500 ppm DEA were tested for their ability to create false positive results.

Organic compounds listed as prioritized chemical contaminants by the US Primary Drinking Water Standards for 1992 (U.S. Environmental Protection Agency, 1991) [20] which contained moieties possibly capable of reacting with DsCl were tested along with three valence-representative toxic metals. These included 2,4-D, methoxychlor, C_5P, 2,4,5-T, barium, lead, and mercury. All tests were performed in triplicate at concentrations ten times greater than their MCL, at their MCL, and at one-tenth their MCL.

Potable water samples were collected from six Texas geographically diverse cities for testing. These cities included Houston, Dallas, Fort Worth, Waco, Nacogdoches, and Hewitt. These samples were then spiked with 1 ppb C_5P and retested according to procedures outlined previously.

A Hewlett-Packard GC/MS (MSD-5970A containing a 9133 XV data station) was used to screen samples of C_5P for other CPs and 2,4-D, methoxychlor, and 2,4,5-T for phenolic impurities. In this method, test samples were diluted by 100 × in anhydrous acetone and 0.5 μl samples were injected onto a crosslinked methylsilicone capillary column (12 m × 0.2 mm) held initially at a temperature of 40 °C. The temperature was then ramped at 20 °C per min to achieve a maximum temperature of 300 °C. A total run time of 20 min was completed to obtain the total ion chromatogram (TIC). Selected ion monitoring was not utilized due to a tendency to detect artifact ions.

A Perkin–Elmer (Norwalk, CT) Fourier Transform Infrared Spectrophotometer (1600 Series) was used to obtain the IR spectra of the unreacted C_5P, 2,4-D, methoxychlor, and 2,4,5-T and the DsCl reaction products of these compounds. A ZnSe cell was used with anhydrous acetone as the solvent. All solvent peaks were subtracted from the IR spectra. All spectra represented 264 scans per test sample. Briefly, 200 μl of DsCl solution were added to 20 μl of test samples. Next 20 μl of sodium carbonate solution was added and the mixture was thoroughly agitated. 1 ml of sodium hydroxide was added and the solution was extracted with methylene chloride. The cell was filled with this extract by capillary action and spectra were obtained for all test samples by scanning %T from 4200 to 400 cm^{-1}.

The stability of fluorescence for this assay was tested. Dansylated 1 ppm C_5P was placed on six minicolumns using the assay procedure. Three columns were stored at ambient temperature and three were stored at 4 °C. The minicolumns stored at ambient temperature were observed every hour for decreasing fluorescence. The minicolumns stored at 4 °C were observed at 24 h intervals.

3. Results

In testing for water-solvated CPs, a bright fluorescent band was defined as a positive test. A very dim fluorescent band, or no detectable fluorescence at the target

interface, was defined as a negative test. It was not our goal to quantitate the intensity of fluorescence.

Our results indicated that the assay was capable of rapidly screening water samples and detecting CP contamination at very low concentrations. Table 1 contains assay results for the CPs. Also, most of the observable error from these studies was caused by false positive results, and in these cases, the assay was detecting CPs at a level below that established as an approximate LOD. Actual failure to detect CPs in water was minimal. Since the assay will be used in the field to screen potable water samples for CPs and is not designed to replace standard analytical techniques, the failure rate was acceptable.

The EPA considers pentachlorophenol (C_5P) to be the most toxic of the CPs. The reaction of a CP and DsCl is kinetically controlled by the rate of formation of the chlorophenate anion. Chlorine is very electronegative and promotes the formation of the chlorophenate anion, such that the rate of formation is: penta- > tetra- > tri- > di- > monochlorophenate. For this reason, the assay was most sensitive to C_5P and the tetrachlorophenols. The EPA has established a maximum contaminant level (MCL) of 1 ppb for C_5P in drinking water. This new assay was able to easily detect

Table 1
Approximate limits of detection and error analyses for the chlorophenol minicolumn assay

Chlorophenol	LOD[a]	Percent error[b] by CP	Percent error by CP class[c]
2-CP	⩾ 1 ppm	11.1	
3-CP	⩾ 1 ppm	11.1	
4-CP	⩾ 1 ppm	0	3.7
2,3-C_2P	⩾ 1 ppm	0	
2,4-C_2P	⩾ 1 ppm	0	
2,5-C_2P	⩾ 1 ppm	0	
2,6-C_2P	⩾ 1 ppm	22.2	
3,4-C_2P	⩾ 1 ppm	0	
3,5-C_2P	⩾ 1 ppm	11.1	5.6
2,3,4-C_3P	⩾ 1 ppb	22.2	
2,3,5-C_3P	⩾ 1 ppb	11.1	
2,3,6-C_3P	⩾ 1 ppb	33.3	
2,4,5-C_3P	⩾ 1 ppb	33.3	
2,4,6-C_3P	⩾ 1 ppb	11.1	22.2
2,3,4,6-C_4P	0.1–1 ppb	22.2	
2,3,5,6-C_4P	0.1–1 ppb	22.2	22.2
2,3,4,5,6-C_5P	0.1 ppb	22.2	22.2

[a] The approximate limit of detection (LOD), in the range of ppm and ppb, was determined for each positional isomer of chlorophenol (CP). Triplicate positive assays were required to establish LOD.

[b] The percent error was established for each CP based on $(x_a - x_b/x_a)\,100$; where x_a is the expected result and x_b is the actual test result. Each CP was assayed in triplicate at 10 × the LOD, the LOD, and 0.10 × the LOD. The average percent error (based on nine assays per CP) was reported.

[c] Five (5) classes of CPs were delineated by the degree of chlorination (i.e., CP, C_2P, C_3P, C_4P, and C_5P). The percent error for CPs by class was obtained by averaging the error for individual CPs in the class.

this level, with no false negative results when run in triplicate. Five aliquots of 500 ppm DEA were assayed. With the addition of a preparative layer of phyllosilicate clay (NovaSil™), no false positive results for CPs occurred in the presence of dansylated DEA. This suggests that smaller amines, which can occur as contaminants in water, can be avoided by selective adsorption and immobilization of dansylated impurities on the minicolumn prior to their reaching the reactive interface for dansylated CPs.

In studies to evaluate the specificity of the assay, all organic compounds regulated by the US Primary National Drinking Water standards of 1992 were examined. Those that had functional moieties potentially capable of dansylation as well as representative metals were obtained and tested with this assay. Of those tested, only C_5P and 2,4-D gave positive results at the MCL, while methoxychlor and 2,4,5-T gave positive results at 10 times their MCL. None of the metals (barium, lead, or mercury) yielded positive results. Results are listed in Table 2.

Methoxychlor and 2,4,5-T gave positive results at 10 times their MCL, but not at their MCL. The herbicide 2,4-D gave positive results at its MCL. None of the chemicals tested yielded positive results, except C_5P at one-tenth their MCL. Fourier transform infrared spectrophotometry (FTIR) and the GC/MS demonstrated that the positive results were probably caused by phenolic impurities. Specifically, the 2,4,5-T contained trace amounts of C_5P. The mass spectrum of the 2,4,5-T contained a peak at 266 a.m.u. identical to the one in the mass spectrum of authentic C_5P. The FTIR spectrum of the 2,4,5-T also contained an absorption band centered at 3600 cm^{-1} which is characteristic of a phenolic hydroxyl band which should not be present in

Table 2
Evaluation of selected organic and inorganic chemical contaminants in water for interfererence with the CP minicolumn assay

Contaminant[a]	MCL (mg/l)	Test results (+ or −)		
		MCL[b]	10 × MCL[c]	0.1 × MCL[d]
C_5P	0.001	+	+	+
2,4-D	0.07	+	−	−
Mchlor	0.04	+	+	−
2,4,5-T	0.05	−	+	−
Ba	10	−	−	−
Pb	0.20	−	−	−
Hg	0.02	−	−	−

[a] Selected from chemical contaminants listed in the U.S. EPA Fact Sheet on Primary Drinking Water Standards [20]. C_5P = pentachlorophenol; 2,4-D = 2,4-dichlorophenoxyacetic acid; Mchlor = methoxychlor; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; Ba^{+2} from $BaSO_4$; Pb^{+2} from $Pb(OAc)_2$; Hg^{+} from Hg_2Cl_2.

[b] Results of tests (confirmed in triplicate) at the MCL.

[c] Results of tests (confirmed in triplicate) at 10 times the MCL.

[d] Results of tests (confirmed in triplicate) at 0.1 times the MCL.

pure 2,4,5-T. Methoxychlor possibly contained a tetrachlorophenol. The mass spectrum had an ion at mass 232 which could correspond to a tetratchlorophenol of mass 232. The herbicide 2,4-D possibly contained a dichlorophenol. The mass spectrum contained an ion at mass 162 which could correspond to a dichlorophenol. The IR spectrum contained the phenolic hydroxyl band previously described. Also, the IR spectra of the dansylation products of C_5P, 2,4,5-T, and 2,4-D all show a reduction in the intensity of the phenolic hydroxyl band, suggesting binding of a phenolic impurity by DsCl.

None of the six potable water supplies yielded positive results. Also, following the addition of 1 ppb pentachlorophenol to each water sample, all tested positive.

The fluorescent complex bound at the reactive interface was stable for periods up to eight hours at room temperature or up to two weeks under refrigeration.

4. Discussion

The reaction of DsCl and a CP is an electrophilic reaction of interest, with several characteristics worthy of study. DsCl is a substituted naphthalene with a conjugated π system that is responsible for its fluorescent complexation [21]. This moiety is very reactive in the presence of phenolic hydroxyl groups. Fluorescence occurs when a σ or π electron is excited to a π^* orbital and relaxes, releasing electromagnetic radiation. The lifetime in the excitation manifold is of about 10 ns. DsCl forms fluorescent complexes with centers of high-electron density because of the availability of delocalized electrons.

Addition of minute quantities of acid or large volumes of base quickly quench the fluorescence of the dansylated CPs. Addition of acid may lead to the formation of an iminium ion. The resulting positive charge could serve to withdraw electrons from the π system. It could also be argued that the formation of a N–H bond would force the electrons on nitrogen to contribute to a σ bond, disrupting extended conjugation. The addition of excess base might provide a source of oxygen which could bind to the sulfur in the sulfonyl group. Coordination of sulfur to six atoms would commit all bonding electrons to σ bonds. The actual electronic environment of the dansylated CPs is ideal for fluorescence and is electronically stable. This would explain the stability of the fluorescent complex.

The reactivity of DsCl with centers of electron density makes possible the dansylation of many classes of chemicals. The addition of excess sodium hydroxide destroys many of these complexes, and the water provided by the sodium hydroxide offers an aqueous medium for removal. This simple extraction process severely curtails the possibility of many false positives. The preparative clay layer serves to bind the dansylated amines, probably the most stable of the dansylated co-contaminants. These two steps make the detection of contaminants less likely.

The reaction of DsCl with C_5P is of interest and the binding at the phenolic hydroxyl group can be demonstrated using FTIR. The spectrum of C_5P demonstrates a very strong absorption band for the phenolic hydroxyl group at 3590–3650 cm^{-1}. Upon dansylation, this band reduces drastically in intensity.

The GC/MS was able to demonstrate that 2,4,5-T (quality grade) probably contains trace amounts of C_5P. The methoxychlor (99% purity) was found to contain a potential tetrachlorophenol and 2,4-D (99% purity) was found to possibly contain a dichlorophenol. The phenolic impurities were most likely responsible for the false positive results. This is supported by the fact that a dilution to $\frac{1}{10}$ the MCL, where phenolic impurities would be present in exceedingly minute quantities, yielded negative results (Table 2).

Hopefully, this assay will facilitate the rapid, field-practical analysis of potable water for chlorophenols. This assay is not meant to replace instrumental techniques of identification and quantitation, rather it is meant to supplement these techniques as a screening technique for contamination.

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