WV TAP
WEST VIRGINIA TESTING ASSESSMENT PROJECT

Assessment of Tentatively Identified Compounds in Tap Water Following the January 9 Chemical Spill from Freedom Industries

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Introduction

This report summarizes the development of a sensitive analytical method for 4-MCHM and PPH and the investigation of sources of additional peaks observed on chromatograms from samples collected as part of the WVTAP 10 home study.

Analytical Method Development

Eurofins Lancaster Laboratories Environmental, LLC.(ELLE) developed an analytical technique for the analysis of 4-methyl-1-cyclohexanemethanol (4-MCHM) CAS #34885-03-5 and propylene glycol phenyl ether (PPH) CAS #770-35-4 in potable water. In the absence of applicable toxicological evaluation and assessment with respect to concentrations that will result in negative human health effects, an analytical method that would be able to detect 4-MCHM and PPH at the lowest levels possible using commonly available instrumentation was desirable.

4-MCHM and PPH have very different characteristics. 4-MCHM (MW = 128.21 g/mole) is a colorless liquid with a density of 0.9074 g/ml and a boiling point of 202 °C. However, physiochemical property data for the contaminants spilled into the Elk River remains limited. The solubility of 4-MCHM was estimated by Dr. Kevin West at the University of South Alabama to range from approximately 2,500 mg/l (0°C) to 3,750 mg/l (100°C). This estimate was determined using COSMO-RS (Conductor like Screening Model for Realistic Solvents (3). Commercially available standards consist of a mix of the cis (axial substitution of the 4-methyl group) and trans (equatorial substitution of the 4-methyl group) isomers. The relative concentration of each isomer is not determined or provided in manufacturer's Certificate of Analysis information. PPH (MW = 152.19 g/mole) is a clear, colorless liquid with a density of 1.059 g/ml, a boiling point of 242.7 °C and a 11,000 mg/l water solubility (4).

Based on the aforementioned physical characteristics and chemical similarity to other compounds analyzed by this laboratory, an approach utilizing gas chromatography with mass spectrometry (GC/MS) and organic solvent extraction was used. The sample preparation step generally followed EPA SW-846 Method 3510. In summary, this method calls for the serial extraction of a water sample with methylene chloride or other suitable solvent. Due to the fact that 4-MCHM and PPH were similar to other compounds analyzed under a semivolatiles or extractable organics approach, methylene

chloride was used as the extraction solvent. The methylene chloride solvent fractions from the serial extractions of the water sample were combined and the total solvent volume reduced to a final volume (FV) of 1 milliliter (ml).

The instrumental analysis generally followed EPA SW-846 Method 8270. In summary, this method uses GC/MS instrumentation that is operated in the electron impact (EI) ionization mode. The GC/MS is tuned to decafluorotriphenylphospine (DFTPP) to "standardize" the consistency of the instrumental response. After tuning, the analytical system is then calibrated using a minimum of a 5-point calibration curve. The calibration curve/response is constructed using internal standard calibration. A calibration curve was considered acceptable if the percent relative standard deviation (%RSD) of the relative response factors (RRF) for the 5 or 6 calibration points was < 20%.

Experimental

For the work performed in preparation for the 10 Home Study under WV TAP, 1 liter of water was serially extracted with methylene chloride and the methylene chloride extracts were concentrated to a FV of 1 ml. Prior to the extraction with methylene chloride, a known volume and concentration of surrogate standards were added to each field sample and the associated quality control (QC) samples.

After extraction of the sample and after the methylene chloride extract is reduced to a volume of 1 ml, but prior to instrumental analysis, a known volume and concentration of internal standards were added to each 1 ml methylene chloride extract. The list of surrogate standards and internal standards added to the samples/extracts was the list of compounds typically used for Method 8270 analysis in the environmental industry. The compounds are listed in Table 1.

Table 1: Surrogate and Internal Standards initially used in 4-MCHM/PPH Method.

Surrogate Standards[1]	Internal Standards
2-Fluorophenol	1,4-Dichlorobenzene-d4
Phenol-d6	Naphthalene-d8
Nitrobenzene-d5 *	Acenaphthene-d10
2-Fluorobiphenyl *	Phenanthrene-d10
2,4,6-Tribromophenol	Pyrene-d10
Terphenyl-d14 *	Perylene-d12

Compounds designated with an asterix (*) are base/neutral surrogate standard compounds.

Early in the work it was recognized that one of the surrogate compounds, nitrobenzene-d5, impacted the detection and analysis of 4-MCHM at lower levels. Nitrobenzene-d5 has several secondary ions that are within an atomic mass unit (amu) of the quantification mass for 4-MCHM. This affected the detection of 4-MCHM at very low levels because nitrobenzene-d5 essentially coeluted with 4-MCHM under the chromatographic conditions of analysis and the mass loading of nitrobenzene-d5 was so substantial relative to 4-MCHM. Therefore, going forward into the 10 Home Study, the base/neutral surrogate standard compounds (those designated with an *) were eliminated from the surrogate standard spiking mixture. We also felt that the phenolic compounds remaining in the surrogate standard spiking solution better represented compounds like 4-MCHM and PPH, compounds that had free hydroxyl groups in the chemical structure.

The GCMS instrument was calibrated with six concentrations of calibration standard (Table 2).

Table 2: Calibration levels used for 4-MCHM and PPH.

Calibration Level	Concentration (µg/l)
1	1
2	5
3	10
4	20
5	40
6	60

Note: Concentration listed in µg/l is the concentration as it relates to the concentration in the water sample.

A relative standard deviation (%RSD) of < 20% for the Relative Response Factors (RRFs) of the initial calibration signified a valid, acceptable calibration. The performance of the analytical system was checked every 12 hours by passing a valid DFTPP tune and a continuing calibration check standard (CCV). A CCV was compliant and within specifications if the percent difference of the RRF in the CCV was < 20% of that of the average RRF observed in the initial calibration.

With every extraction group, the following Quality Control (QC) was run. Definitions of appropriate QC terms are shown below.

Extraction Batch – A group of field samples and associated QC extracted with methylene chloride and processed as a group. An extraction batch is not to exceed 20 field samples.

Method Blank – An aliquot of laboratory grade water that is processed through the entire extraction process and is handled (surrogates and internal standards) like a sample. It is used to monitor background contribution of analytical system and process to analytical results.

Laboratory Control Sample (LCS) – An aliquot of laboratory grade water that is spiked with a known quantity of the target compound(s) and processed through the entire extraction process. The spiking concentration is typically at or around the mid-point of the calibration curve. The recovery of the spiked target compound(s) is determined and the efficiency of the extraction process, as it relates to the specific batch, is assessed. Recoveries of 70%-130% were expected for MCHM and PPH. Recoveries outside of the 70%-130% window, particularly below 70% would be cause for the batch to be re-extracted.

Laboratory Control Sample Duplicate (LCSD) – Same as an LCS and when processed in conjunction with an LCS used to measure the precision of the analysis.

Minimum Reporting Limit LCS (MRL LCS) – An LCS for which the concentration at which the LCS is spiked is at or near (typically 1-2x) the minimum reporting limit for the analysis.

Matrix Spike and Matrix Spike Duplicate (MS/MSD) – additional aliquots of a field sample that are spiked, like the LCS, at the mid-point of the calibration curve.

Surrogate Standards – Compounds that are spiked into every sample and that are different from the target compound(s) but expected to extract similarly to the target compound(s). The recovery of the surrogate standards are determined in each sample, which becomes a measure of the efficiency of the extraction for that individual sample.

Internal Standard – Compounds added to the methylene chloride extract prior to instrumental analysis. Internal standards are used to a) monitor the effectiveness of each sample extract injection into the analytical system and b) calculate a response ratio with the target compound(s) in the initial calibration that can be used to quantify target compound(s) in subsequent sample analysis.

The results of the application of EPA Methods 3510 and 8270 towards the analysis of 4-MCHM and PPH are an analytical technique capable of reporting 4-MCHM and PPH to a limit of quantitation (LOQ) of 1 µg/l (ppb) and a method detection limit (MDL) of 0.5 µg/l as shown in Table 3.

Table 3: MDL determination for 4-MCHM and PPH.

Parameter	Compound	
	4-MCHM (µg/l)	**PPH (µg/l)**
MDL1	1.82	1.99
MDL2	1.83	2.00
MDL3	1.83	1.98
MDL4	1.79l	1.95
MDL5	1.83	2.02
MDL6	1.93	2.09
MDL7	1.90	2.03
Mean	1.85	2.01
Spike Level	2.0	2.0
Mean % Recovery	92.4 %	100.5 %
Standard Deviation	0.050	0.046
Statistical MDL*	0.158	0.144

*MDL used for reporting was increased to 0.5 ppb (5).

No preservation other than refrigeration (e.g. acidification or dechlorination) was used for the sample bottles for the 10 home study as it was not clear whether these could interfere with the analysis or react with the target analytes.

10 Home Study Data Review

Analysis of samples from hot and cold water taps at different points throughout each house in the WVTAP 10 Home Study indicated detections of 4-MCHM ranging from just below 1 µg/l to a high of approximately 6 µg/l. 4-MCHM was detected in all samples in all the houses. PPH was not detected in any of the samples collected from the 10 Home Study. An example chromatogram is shown in Figure 1.

The chromatographic peaks for the 6 internal standards and the 3 surrogate standards are listed on the chromatogram. However, a very distinct series of unknown peaks were also detected in the samples. The chromatogram presented in Figure 2 is an overlay of the chromatogram generated from the 4-MCHM analysis of a hot and cold water tap at 2 different houses in the 10 Home Study. The pattern of unidentified peaks detected in each sample was very similar if not the same.

A rough estimation from the visual observation of the chromatograms suggested concentrations for the unknown peaks/compounds in the range of 10 µg/l for many of the peaks to almost 200 µg/l for the significant peak observed at approximately 3.3 minutes (time is on the X axis of the chromatograms). Due to the high potential concentrations of these additional peaks it was deemed important to identify them and determine if they could be oxidation or other breakdown products of the 4-MCHM or if they could

represent additional compounds from the spill or were they coming from some other source.

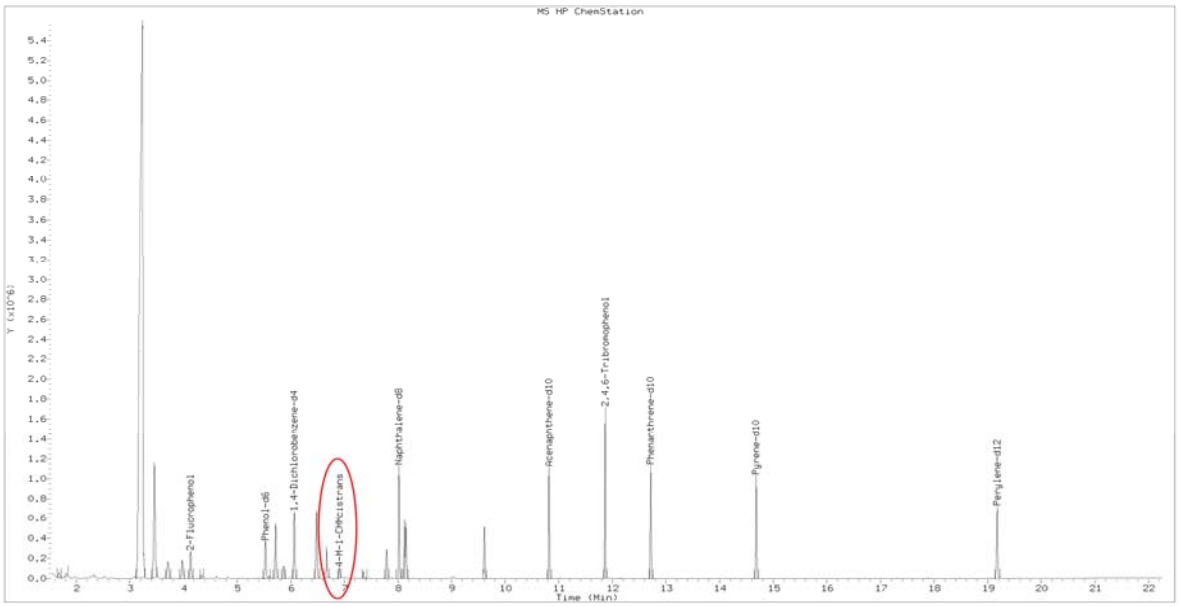

Figure 1: Example total ion chromatogram for one of the 10 house study samples. Red circle shows the peaks and location of 4 – MCHM. Peaks with names are surrogates and internal standards.

A close-up of the peak pattern that was observed is shown in Figure 3.

In an attempt to determine the identity of the unknown peaks, mass spectral library searches were performed on the chromatograms and GC/MS data files for the 10 Home Study samples. A mass spectral library search is a tool used by analytical chemists to attempt to tentatively identify and semi-quantitatively quantify the compound responsible for the observed chromatographic peak. Library search databases are available from standard reference sources like the National Institutes for Standards and Technology (NIST) and are typically part of most GC/MS data systems

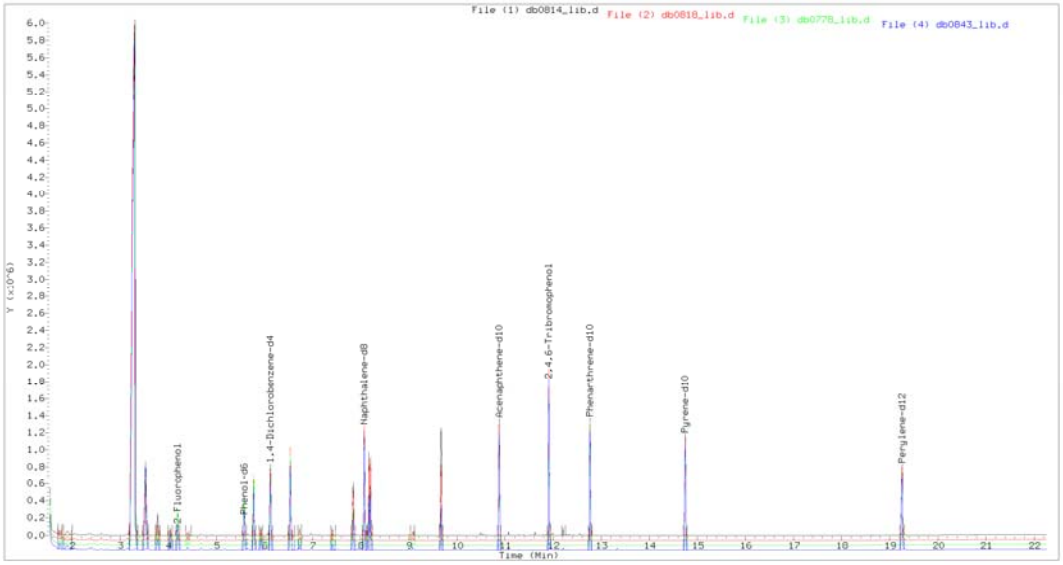

Figure 2: Overlay of chromatograms from multiple samples from 10 home survey. Peaks with names are surrogates and internal standards.

Library search data bases are generally useful when the analytical technique is EI (electron impact) ionization, which under controlled conditions fragments chemical compounds into predictable and relatively consistent ion fragment patterns. Because of the relative predictability of the ion fragment patterns, the ion fragment pattern from an unknown peak can be compared to the library's database of ion fragment patterns, with the intention of matching patterns and potentially identifying the compound responsible for the unknown peak. As the computer software that performs this function operates, it also assigns a quality of match number between the unknown compound ion fragment and the library database reference compound ion fragment. This quality of match indicator is typically on a scale of 0-100. The closer the number is to 100, the better the match between the unknown and the database reference compound.

The result of a library search on a given sample is a list of possible matches, called Tentatively Identified Compounds (TIC), a quality of match value and an estimated concentration. The estimated concentration is a very gross estimation in that it is calculated by using the response factor for an internal standard used in the sample analysis, to quantify the Tentatively Identified Compound (TIC).

For the 10 Home Study, library searches were performed on the GC/MS data file generated from the analysis of the cold water kitchen tap and the hot water kitchen tap for each house. Table 4 summarizes the most prevalent identifications listed in the TIC library search results. This is not a comprehensive list, but is presented as representative of what was "detected" and the tentative identifications assigned to them.

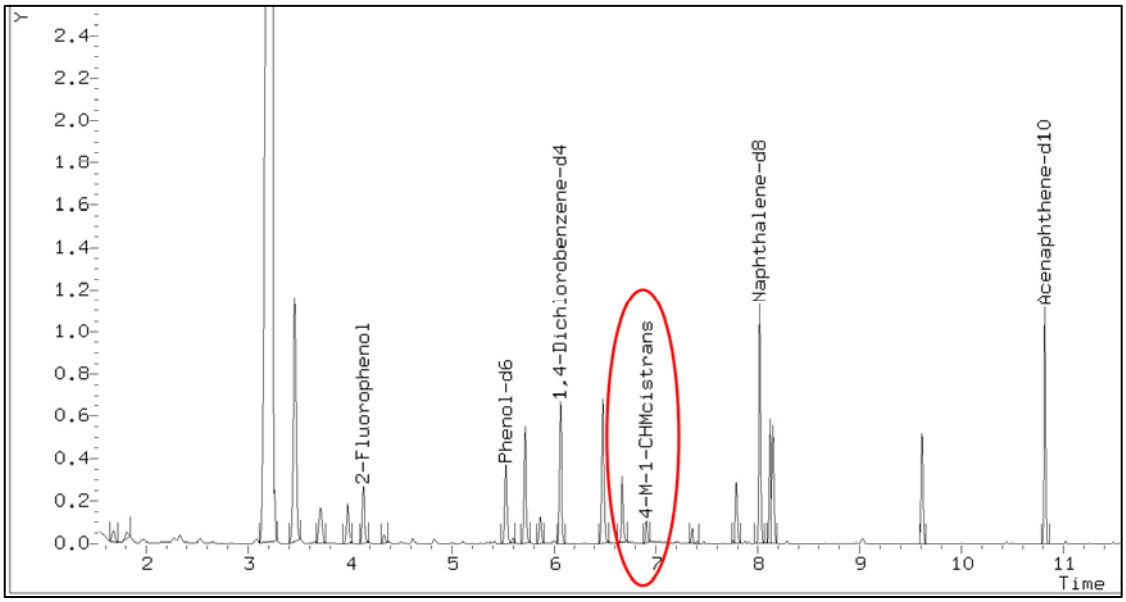

Figure 3: Expanded view of chromatogram showing additional peaks besides the surrogates, internal standards, and target compound. Peaks with names are surrogates and internal standard

Table 4: Most prevalent TIC identifications from library search of unknown compound peaks.

CAS Number	Compound Name	RT, min.	Estimated Conc., µg/l (rounded)
17773-64-7	1-Butene, 2-chloro-3-methly-	1.892	1.4
1985-88-2	1,1-Dimethyl-3-chloropropanol	3.309	200
507-45-9	Butane, 2,3-dichloro-2-methyl-	3.530	13.6
2419-74-1	2-Butanol, 1,4-dichloro-	3.781	3.7
74421-00-4	Butane, 2,3-dimethoxy-2-methyl	4.043	1.0
0-00-0	O-chlorophenol-d4	5.774	6.5
27639-93-9	Propanoic acid, 2-chloro-	6.538	9.3
77-73-6	4,7-Methano-1H-indene, 3a,4,7,7a-tetrahydro-	7.843	4.0
392-71-2	2,6-Dichloro-4-fluorophenol	8.170	5.8
21031-46-9	3-Butenenitrile, 3-chloro-	8.205	5.6
10025-67-9	Sulfur monochloride	9.674	9.3

We observed the following features for these TICS.

a. All of the houses tested showed these same unidentified peaks with the exception of one house that had low chlorine residual in field measurements. That house did not have any of the TICs
b. The TICs were processed against several different libraries that were available to the team and they generally produced the same identifications.
c. None of the TICs found in the house samples were observed in our analysis of the crude MCHM, supplied by Dr. Michael McGuire from samples obtained from the West Virginia National Guard from the material in the Freedom spill.
d. The TICs are really only presumptive positive detections, so to accurately designate the identity of a compound would require that an analytical grade standard of the presumptive compound be obtained and analyzed under the conditions of the GC/MS analysis. Only if the chromatographic retention time and the mass spectral ion fragmentation matched would chemists be able to positively identify the compound.
e. The TIC at 3.5 minutes, 2,3-dichloro-2-methylbutane, has been proposed to be a by-product of reactions between plastic pipes and chlorine by others[2] and therefore is likely not related to the crude MCHM.
f. At least two of the TIC peaks appeared to be deuterated chlorophenols, that is chlorophenols containing a different form of hydrogen, such as was found in the surrogates that are added by chemists as part of the sample preparation process for analysis.
g. The peak at 9.674 minutes, identified by the library search as sulfur monochloride, actually matched well with that of 2,4,6-trichlorophenol-d2, even though the library search database was not able to distinguish this compound.

The observation that two of the TICs appeared to be deuterated compounds was a concern. There was not an obvious scenario under which we would have expected to have detected deuterated compounds at the estimated concentrations listed. We subsequently confirmed the identities of the peak at 5.74 minutes (o-chlorophenol-d4) and the one at 9.674 minutes (2,4,6-trichlorophenol-d2) by obtaining standards of these compounds and matching retention times and spectra.

The only obvious source of deuterated compounds was from the surrogate standard mix mentioned previously. Since these two TICs were identified as phenolic type compounds and phenol-d6 and 2-fluorophenol are both phenolic type surrogates, we suspected these might be the source.

An experiment was performed to determine if these compounds, the deuterated ones in particular, were the result of a reaction with the surrogate compounds listed. To evaluate if this reaction was the cause, a sample of water from ones of the houses was spiked with surrogate compounds and extracted/analyzed under the normal set of analytical conditions. A second aliquot of water from the same house was not spiked with the surrogates and then extracted and analyzed.

The chromatograms are shown in Figures 4 and 5. Figure 4 shows the house sample that had the surrogate standard mix added prior to extraction. The chromatogram shows the surrogate standards and internal standards (all labeled) and the tentatively identified compound pattern that has been described previously. Figure 5 shows the internal standards (labeled), no surrogates (not added) and virtually all of the tentatively identified compounds are missing. The large peak at 3.3 minutes and the pipe reaction product at 3.5 minutes remain. Therefore, it appeared that the presence of the TICs was in fact a reaction between two of the surrogate standard compounds, phenol-d6 and 2-fluorophenol, and residual chlorine in the water. This was based on the tentatively identified compound names, which were in most cases some version of a chlorinated phenol.

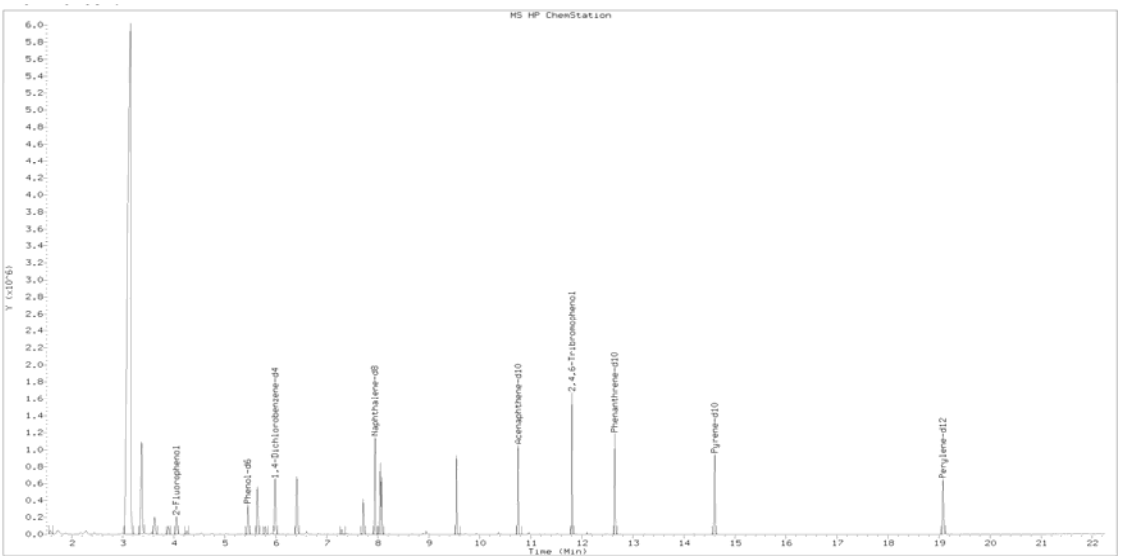

Figure 4: Chromatogram with surrogate standards added. Peaks with names are surrogates and internal standards.

This hypothesis was also consistent with the observation that these peaks were not present in the one house sample that had very low residual chlorine in the field testing. These peaks therefore are considered artifacts of the analytical process and are in no way related to the MCHM spill.

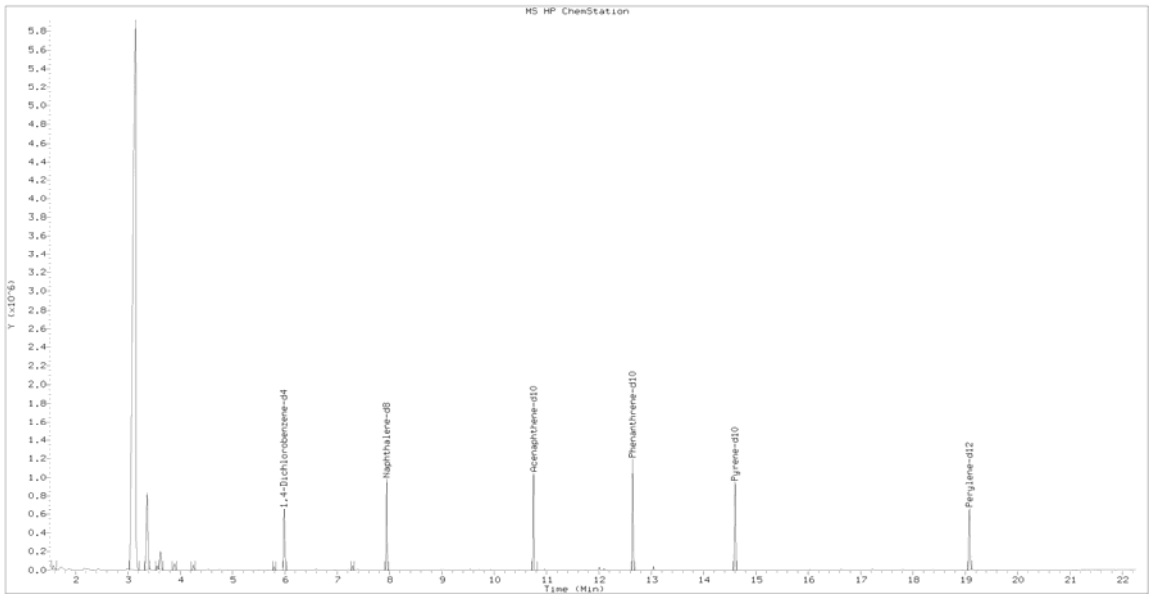

Figure 5: Chromatogram with surrogate standards not added. Peaks with names are internal standards.

To further validate this conclusion, two additional aliquots of water from one of the houses were obtained. One aliquot was dechlorinated with sodium sulfite and the other was not. Both aliquots had surrogate standards added to them prior to being extracted and analyzed by the 4-MCHM method. The chlorinated water displayed the TICs that had been observed in all of the 10 Home Study samples. The dechlorinated water (Figure 6) did not show any of the TICs. In fact, the dechlorinated water also did not exhibit the large peak at 3.3 minutes.

We also verified that the TICs were not related to the MCHM by taking an aliquot of Lancaster PA tap water, adding additional chlorine, and extracting and analyzing it using the method described here, including all of the surrogates. No 4-MCHM was detected, but the same tentatively identified compounds were observed. A second aliquot was dechlorinated and extracted. No tentatively identified compound peaks were observed.

Prior to the experiment with water dechlorination, efforts were undertaken to determine the identity of the large tentatively identified compound observed at approximately 3.3 minutes in all of the samples from the 10 Home Study. The presumptive identification of this peak when compared to several libraries consistently was identified as 1,1-dimethyl-3-chloropropanol. The analytical grade reference material was acquired and analyzed under the set of GC/MS conditions used for the analysis of 4-MCHM. The mass spectral

match was relatively good with that observed for the TIC, however, the retention time of the 1,1-dimethyl-3-chloropropanol was approximately 2 minutes later than that observed for the large tentatively identified compound, indicating that it could not be the compound identified in the library search. To further confirm that this was not some form of retention time shift, the extract of the house sample was spiked with the reference material and it showed as a clear second peak on the chromatograms.

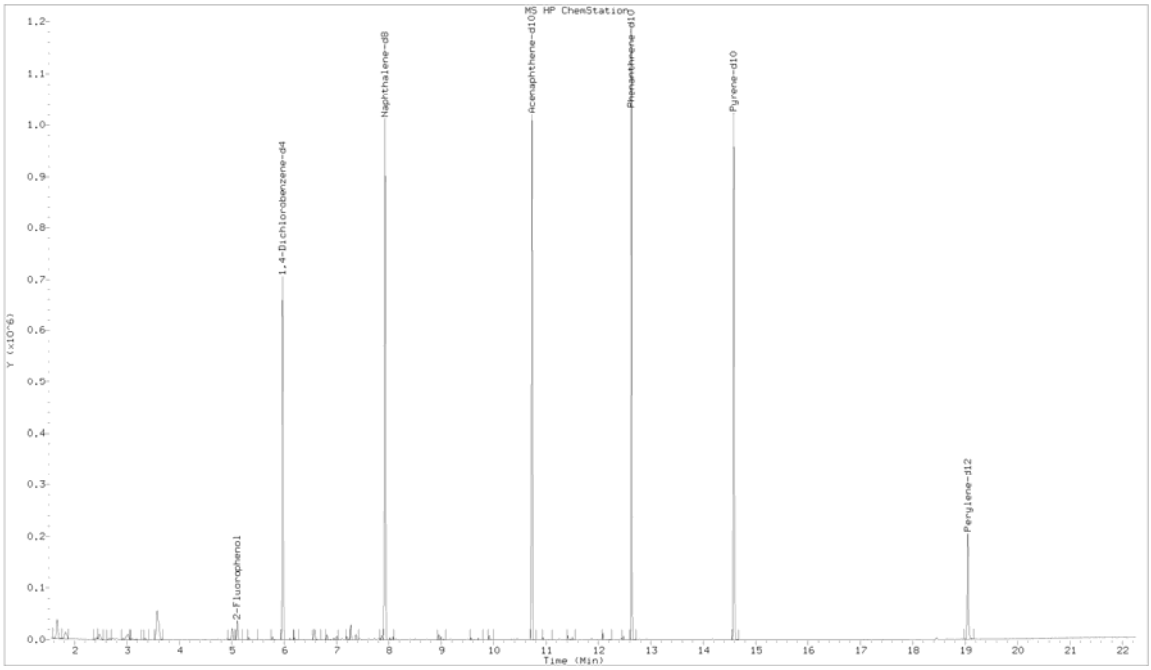

Figure 6: Dechlorinated house water – sodium sulfite added Peaks with names are internal standards.

This prompted an investigation as to whether or not there was something else in the water, unrelated to the crude MCHM which could be the source of the peak. To follow this line of reasoning, four (4) additional samples were collected upstream of the Freedom Industries spill site, at the West Virginia American Water (WVAW) facility influent, at the WVAW effluent, and from a house. An aliquot of water from each of these four sampling points was extracted and analyzed by the 4-MCHM analytical procedure. Neither 4-MCHM nor the large TIC at 3.3 minutes were detected in the upstream or influent sample, but both were detected in the effluent sample and the house sample. When this information was considered along with the results of the surrogate standard/chlorination work, it was postulated that the large TIC at 3.3 minutes was likely some sort of disinfection by-product.

A review of literature on disinfection by-products uncovered an article in the International Journal of Spectroscopy, by Karl J. Jobst and Johan K. Terlouw from

McMaster University in Ontario, Canada (1). In this article the authors identified some "disinfection" by-products that are actually the result of the reaction of chlorine in water samples with preservatives used in the manufacture of the methylene chloride which is used as part of the analytical method for extracting these water samples. The spectra and relative retention time information provided in the article matched what we observed in the 10 Home Study samples very well. We confirmed that the preservative suspected of reacting with the chlorine in the water, 2-methyl-2-butene, is in fact the stabilizer used in the methylene chloride used for analysis of samples in the 10 Home Study. Therefore, we would expect that if the water is dechlorinated prior to extraction, the large TIC at 3.3 minutes would not be present. That was confirmed in the dechlorination work mentioned previously.

We also examined chromatograms from the second laboratory participating in the 10 Home Study and saw that the large peak was also present in their chromatograms along with some of the other TICs we identified. This was initially a cause for concern as the lab reported that their sample bottles contained sodium thiosulfate, a dechlorinating agent, and we would therefore have expected to see no peaks that were related to chlorine. However upon query of the laboratory we determined that the amount of thiosulfate that was added to their bottles was 10 mg/l, which is well below the level normally used for dechlorination of drinking water samples (40-80 mg/l) for analysis of semivolatile compounds. The lab also reported low recoveries of some of their surrogates, which is consistent with reaction with chlorine. Thus we were confident that there was no inconsistency in TIC results between there chromatograms and ours.

Conclusion

Numerous tentatively identified compounds observed in the samples analyzed in the WVTAP 10 Home Study were created as a result of the reaction of the chlorine in the treated water with;

a. Several surrogate standard compounds routinely used in 8270 analysis
b. One of the stabilizers used in the manufacture of methylene chloride, which is the solvent of choice for most 8270 type analyses.

There is no evidence presented here or in the course of the analysis of the 10 home study water samples, that would indicate that during mid-February, more than 1 month after the spill, the crude MCHM contributed to the creation or presence of the observed tentatively identified compounds. Our conclusions are that there were no breakdown compounds related to the MCHM spill that could be measured when these samples were collected, at the detection levels attained in this study (which were very low).

Additionally there is no evidence that the presence of chlorine in the samples interferes with the analysis of 4-MCHM or PPH. However, future sampling should include adequate amounts of dechlorinating agents to minimize the occurrence of tentatively identified compounds that are the result of reactions with chlorine.

References

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accessed 5/20/14